

09 – 11 October **2023**

Zoonoses

International
Symposium

on Zoonoses Research

Benefits and Chances
of One Health Research

Program and
Abstracts



Funded by
Federal Ministry
of Education
and Research



EUROPÄISCHE UNION
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Dieses Vorhaben wurde als Teil der Reaktion der Union auf die Covid-19-Pandemie finanziert.

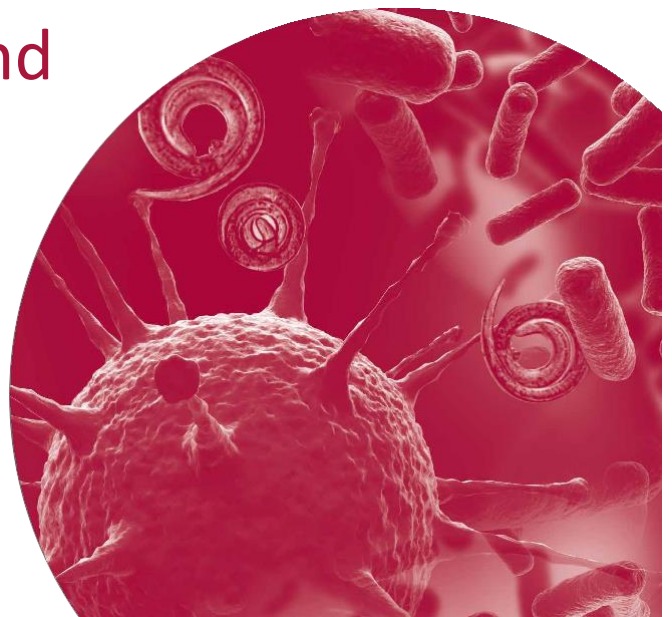


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Program

Monday, October 9th, 2023

13:00	Registration / Check In	
	<i>Atrium</i>	13:00 - 14:00
14:00	Welcome Note	
	<i>MOA 4+5</i>	14:00 - 14:30
	Keynote 1: Göran Kauermann (LMU München)	
	<i>MOA 4+5</i>	14:30 - 15:00
15:00	Break	
	<i>Atrium</i>	15:00 - 15:30
16:00	Session 1: Epidemiology and Secondary Data Use	Session 2: Vaccines & Immunology
	<i>MOA 4+5</i> 15:30 - 17:00	<i>MOA 6</i> 15:30 - 17:00
17:00	Coffee Break	
	<i>Atrium</i>	17:00 - 17:30
18:00	One Health Pioneers: Appreciation Thomas C. Mettenleiter & Lothar Wieler	
	<i>MOA 4+5</i>	17:30 - 18:30
19:00	Session 3: Pathogenesis & Pathology of Zoonotic Infections	Session 4: Host-pathogen Interactions 1
	<i>MOA 4+5</i> 18:30 - 20:00	<i>MOA 6</i> 18:30 - 20:00
20:00	Poster Slam	
	<i>MOA 4+5</i>	20:00 - 21:00
21:00	Get-Together & Poster Viewing (P1)	
	<i>Atrium</i>	21:00 - 22:00

Tuesday, October 10th, 2023

10:00

Keynote 2: Raina Plowright (Department of Public & Ecosystem Health, Cornell University College of Veterinary Medicine, New York)

11:00

Session 5: Environmental factors & Ecology of Zoonotic Infections

Session 6: Public Health & Pandemic Preparedness

12:00

MOA 4+5

11:00 - 12:30

MOA 6

11:00 - 12:30

Coffee Break

Atrium

12:30 - 13:00

13:00

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity

Session 8: Antimicrobial Use and Resistance

14:00

MOA 4+5

13:00 - 14:30

MOA 6

13:00 - 14:30

Lunch & Poster Viewing (P2)

15:00

Atrium

14:30 - 15:30

Keynote 3: Timo Falkenberg (GeoHealth Centre, UK Bonn)

MOA 4+5

15:30 - 16:00

16:00

Coffee Break

Atrium

16:00 - 16:30

Members Assembly of the German Research Platform for Zoonoses with Election of the Internal Advisory Board

17:00

18:00

19:00

MOA 4+5

16:30 - 20:00

20:00

Social Dinner

21:00

MOA 8+9

20:00 - 22:00

22:00

Wednesday, October 11th, 2023

09:00	Young Scientist Breakfast	
10:00	<i>MOA Eat (restaurant of the event's venue site)</i> 09:00 - 10:30	
11:00	Session 9: Zoonoses & Wildlife I	Session 10: Bioinformatics, Digitalization and AI In One Health Research
12:00	<i>MOA 4+5</i> 10:30 - 12:00	<i>MOA 6</i> 10:30 - 12:00
12:00	Coffee Break	
13:00	<i>Atrium</i> 12:00 - 12:30	
13:00	Session 11: Host-pathogen Interactions 2	Session 12: Zoonoses & Wildlife II
14:00	<i>MOA 4+5</i> 12:30 - 14:00	<i>MOA 6</i> 12:30 - 14:00
14:00	Lunch	
15:00	<i>Atrium</i> 14:00 - 15:00	
15:00	Keynote 4: Marlon Koopmans (Erasmus MC)	
16:00	<i>MOA 4+5</i> 15:00 - 15:30	
	Poster Prize	
	<i>MOA 4+5</i> 15:30 - 16:30	

General information

Date and Venue October 9-11, 2023

Mercure Hotel MOA Berlin
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10559 Berlin

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Conference Languages
The official conference languages is English.

Steering Committee
Martin H. Groschup (Greifswald - Insel Riems)
Stephan Ludwig (Münster)
Christian Drosten (Berlin)

Organization
Office of the German Research Platform for Zoonoses

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Research Network of Zoonotic Infectious Diseases
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Poster Prize

All participants of the meeting are encouraged to elect the three best posters with poster slam videos presented by Junior Scientists at the symposium. The vote will take place online and will reflect the final status of the submitted poster slam videos. Posters that will not be listed will not be available for voting. You will find the link to the vote at the registration desk. The voting will be closed on Friday at 1 p.m. The winners will be announced after “Keynote 4: Marion Koopmans (Erasmus MC)”.

Keynote Speakers

Dr. Timo Falkenberg (Institut für Hygiene und Public Health, GeoHealth Centre)
Prof. Dr. Göran Kauermann (Ludwig-Maximilians-Universität München)
Prof. Dr. Marion Koopmans (Erasmus MC)
Dr. Raina Plowright (Department of Public & Ecosystem Health, Cornell University College of Veterinary Medicine, New York)

Poster Presentations / Poster Slam

Posters will be presented during all three days of the conference. The posters will be allocated in the Atrium and grouped by topics. The posters of Junior Scientists which also appear in the Poster Slams will be marked on site.

In this book of abstracts they are marked with this symbol:



This year’s Poster Slam is scheduled as following:

Monday, October 9th, 20:00 – 21:00

Session 1: Epidemiology and Secondary Data Use
#330

Session 2: Vaccines & Immunology
#256, #301

Session 3: Pathogenesis & Pathology of Zoonotic Infections
#195, #204, #214, #231

Session 4: Host-pathogen Interactions
#177, #180, #192, #194, #201, #210, #236, #250, #251, #263, #265, #266, #267, #282, #283, #292, #315

Session 5: Environmental factors & Ecology of Zoonotic Infections
#217, #253, #275, #325, #326

Session 6: Public Health & Pandemic Preparedness
#252, #285, #321

Session 8: Antimicrobial Use & Resistance
#199, #216, #220, #259, #318

Session 9: Zoonoses & Wildlife
#186, #189, #268, #271, #303, #314, #324

Funding

The International Symposium on Zoonoses Research (Zoonoses 2023) is funded by the European Union, the Federal Ministry of Education and Research and sponsored by Pfizer.



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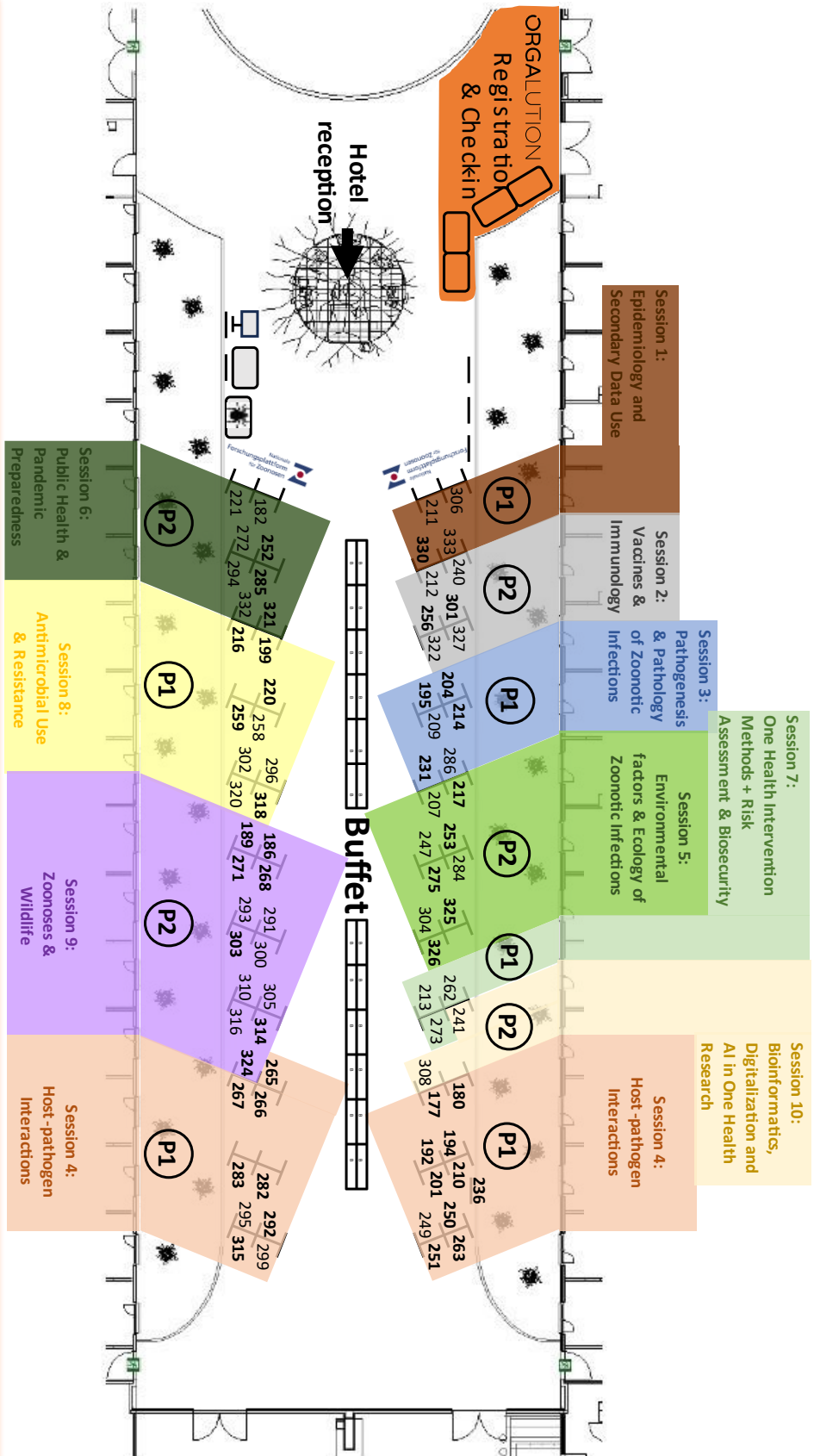
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Dieses Vorhaben wurde als Teil der Reaktion der Union auf die Covid-19-Pandemie finanziert.



Floor Plan

Atrium (2nd floor) • Poster exhibition / Buffet



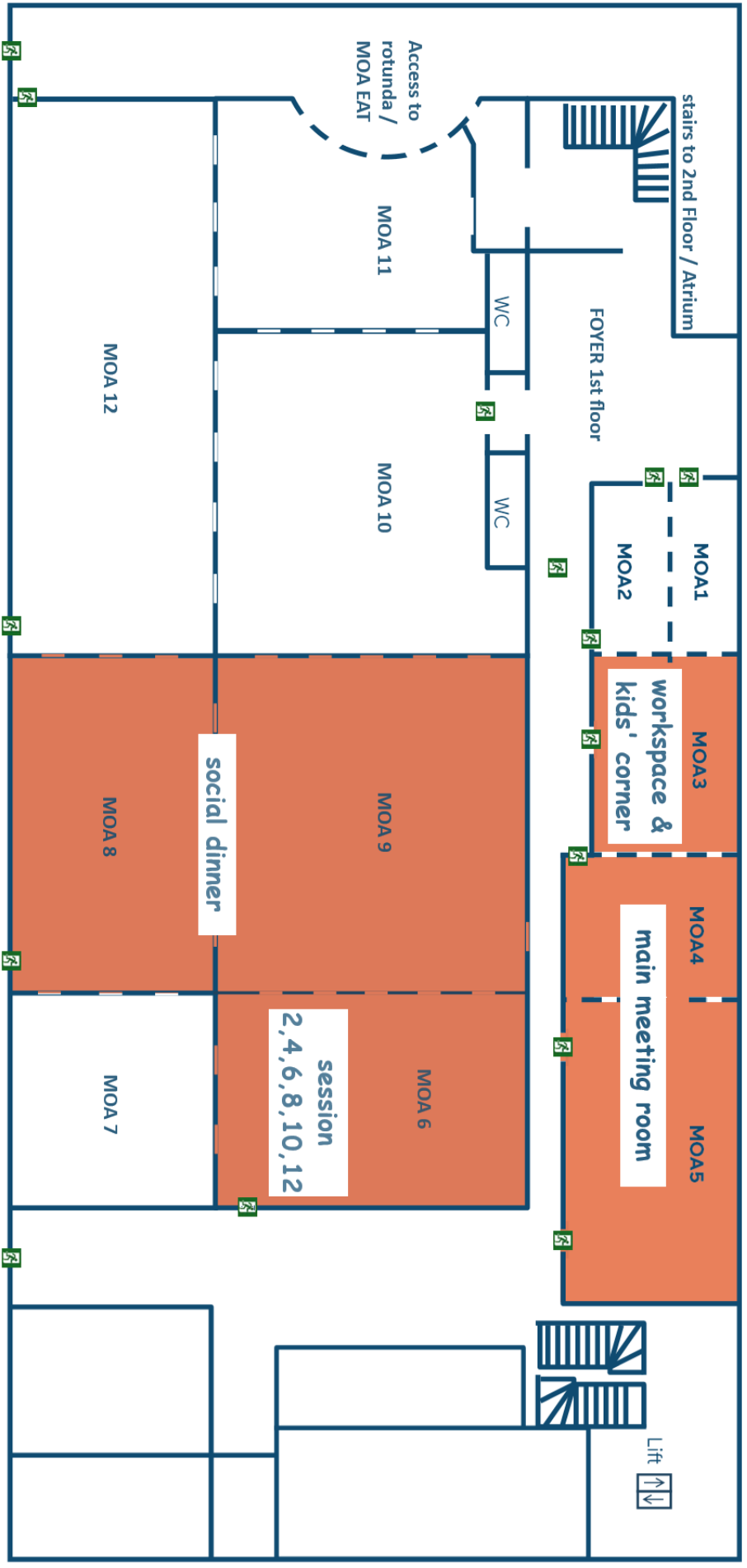
Explanation • Numbers, e. g. 306 = Abstract-ID

P1 = Poster Viewing 1: Monday, 09.10.2023, 9 - 10 p.m.

P2 = Poster Viewing 2: Tuesday, 10.10.2023, 2.30–3.30 p.m.

• Bold, e. g. 330 = Posters of Junior Scientists with Poster Slam Video

MOA 3 - 9 (1st floor) • Sessions / Workspace / Social Dinner



About the German Research Platform for Zoonoses

The German Research Platform for Zoonoses is a central information and service network, initiated and funded by the German Federal Ministry of Education and Research (BMBF) in 2009, for all working groups operating in Germany in the field of zoonoses research.

The objective of the platform and its currently over 1300 members is to increase the exchange of professional experiences and knowledge at national and international levels and thus intensify research activities in the field of zoonoses research, promoting broad horizontal cross-linking of human and veterinary medicine as well as other scientific disciplines related to zoonotic disease research and public and veterinary health services. To develop and maintain sustainable and flexible solutions strengthening research, prevention and therapy of zoonotic infectious diseases in Germany, the Research Platform offers the following measures:

- Organization and realization of joint events that support interdisciplinary exchange and interaction.
- Encouragement of communication as well as national, European and international collaboration.
- Registration, harmonization and standardization of existing resources, including the setting up of both real and virtual specimen databases (i.e. the Database Internet Portal)
- Providing information about zoonotic infectious diseases for the general public
- Initiation and realization of innovative and interdisciplinary research projects (pilot projects, cross-sectional projects, interdisciplinary doctoral projects)
- Support and counseling for the design and implementation of zoonotic funding schemes
- Furtherance of junior scientists in the field of zoonosis research

Acting as a central service point that provides fact-oriented, transparent information relating to research on zoonoses both for politics and the general public, the German Research Platform aims to be the definite voice of German zoonosis research. Additionally, the platform also promotes a continuous and intensive exchange of expertise between scientists from all over the world. Since 2017 it houses the Research Network of Zoonotic Infectious Diseases with seven large research networks and six junior research groups.

As part of these activities, the German Research Platform for Zoonoses organizes every year the National Symposium on Zoonoses Research with up to 500 participants.

Furthermore, scientific workshops, also for researchers at the beginning of their career, are organised, where specific topics are presented and discussed.

All researchers working on zoonoses in Germany are welcomed to join the German Research Platform for Zoonoses.

For further information please visit our website www.zoonosen.net or contact us via email (info@zoonosen.net).

Welcome Note of the German Research Platform for Zoonoses

Authors: Martin H. Groschup¹; Stephan Ludwig²

¹ *Greifswald, Insel Riems*

² *Münster*

Dear colleagues,

Science is a highly dynamic field, always striving to set new standards and create new opportunities for the future. Consequently, the German Research Platform for Zoonoses has never stood still since its foundation in 2009 and has continuously evolved.

Now, a major transformation step is imminent: the German Research Platform for Zoonoses is expanding into a One Health Platform. This step will allow us to have an even broader perspective on human, animal and environmental health research in the future and to better integrate and network the German zoonoses research community, which has grown over the years, into interdisciplinary research initiatives at the national and international level.

Major societal challenges, such as climate change, a growing world population and the loss of biodiversity, also pose new demands on infectious disease research. The stronger integration of zoonoses research in a One Health context offers numerous new opportunities. In particular, better linkages with environmental and social sciences will enable new approaches to the control and prevention of health risks to humans, animals, and the environment.

The interdisciplinary zoonoses research community in Germany has steadily developed over the past years and represents a powerful research community with more than 1300 members. For this reason, it is only logical that it should form the basis and core for the new One Health Platform. Thus, (zoonotic) infectious diseases and antimicrobial resistance (AMR) as well as their interactions with climate, environment and biodiversity will (initially) remain the core topic of the new One Health Platform.

This year's symposium "Zoonoses 2023" is thus the last of its kind. At the same time, it already forms the bridge towards the new One Health Platform, which is also reflected in this year's leading theme of the event "Chances and Benefits of One Health".

The success of the Zoonoses Platform is based on its committed members. We hope to continue this fruitful collaboration in the future and to contribute together to the sustainable establishment of a strong, networked One Health research landscape. The content of the new One Health Platform will be based on the needs and impulses from its foundation - its members. You are therefore cordially invited to help shaping the new One Health Platform and fill it with life.

We look forward to the new opportunities arising from the transformation process and to an exciting last "International Symposium on Zoonoses Research" with you!

Liebe Kolleginnen,

die Wissenschaft ist ein hoch dynamisches Feld, welches stets bestrebt ist, neue Standards zu setzen und neue Möglichkeiten für die Zukunft zu schaffen. Folgerichtig stand auch die Nationale Forschungsplattform für Zoonosen seit ihrer Gründung 2009 niemals still und hat sich kontinuierlich weiterentwickelt. Nun steht ein weitreichender Veränderungsschritt an: die Nationale Forschungsplattform für Zoonosen erweitert sich zu einer One Health Plattform. Dieser Schritt ermöglicht es uns, zukünftig die Gesundheitsforschung für Mensch, Tier und Umwelt aus einer noch breiten Perspektive zu betrachten und die über die Jahre gewachsene Zoonosenforschungs-Community Deutschlands besser in (inter-)nationale fächerübergreifende Forschungsinitiativen einzubinden und zu vernetzen.

Große gesellschaftliche Herausforderungen, wie der Klimawandel, eine wachsende Weltbevölkerung und der Verlust von Biodiversität, stellen auch neue Aufgaben an die infektionsmedizinische Forschung. Die stärkere Einbindung der Zoonosenforschung in einen One Health Kontext bietet dabei zahlreiche neue Möglichkeiten. Insbesondere die bessere Vernetzung mit Umwelt- und Sozialwissenschaften ermöglicht neue Ansätze bei der Bekämpfung und Prävention von Gesundheitsrisiken für Mensch, Tier und Umwelt. Die interdisziplinäre Vernetzung der Zoonosenforschungs-Community in Deutschland hat sich über die letzten Jahre stetig weiterentwickelt und stellt eine leistungsstarke Forschungsgemeinschaft mit mehr als 1300 Mitgliedern dar. Aus diesem Grund ist es nur folgerichtig, dass sie die Grundlage und den Kern

für die neue One Health Plattform bilden soll. Damit bleiben (zoonotische) Infektionskrankheiten und antimikrobiellen Resistenzen (AMR) sowie deren Interaktionen mit Klima, Umwelt und Biodiversität (zunächst) das Kernthema der neuen One Health Plattform.

Das diesjährige Symposium „Zoonoses 2023“ ist damit das letzte seiner Art. Gleichzeitig bildet es bereits die Brücke hin zur neuen One Health Plattform, was sich auch im diesjährigen Leitthema der Veranstaltung „Chances and Benefits of One Health“ widerspiegelt.

Der Erfolg der Zoonosenplattform gründet sich auf ihren engagierten Mitgliedern. Wir hoffen auch in Zukunft auf diese fruchtbare Zusammenarbeit zählen zu können und gemeinsam mit Ihnen einen Beitrag zur nachhaltigen Etablierung einer leistungsstarken, vernetzten One Health Forschungslandschaft beitragen zu können. Auch die neue Plattform wird sich inhaltlich an den Bedürfnissen und den Impulsen aus ihrer Basis - ihrer Mitgliedschaft - orientieren. Sie sind also herzlich eingeladen die neue One Health Plattform mitzugestalten.

Wir freuen uns auf die neuen Möglichkeiten, die sich durch den Transformationsprozess ergeben und auf eine spannende Veranstaltung mit Ihnen!

Welcome Note of the Federal Government

Ladies and Gentlemen,
Colleagues,

I am delighted to be able to welcome you on behalf of the ministries that are involved in the National Research Platform for Zoonoses, namely the ministries of education and research, health, defence and food and agriculture, to the National Research Platform for Zoonoses' 16th and final International Symposium for Zoonoses Research. The subject of this year's symposium, "Benefit and Chances of One Health Research" refers to the logical development of the Research Platform for Zoonoses into a research platform for One Health. This means that the Ministry for Economic Cooperation and Development and the Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection are also part of the research agreement and consequently also part of the research platform for One Health. This event is therefore not just a conclusion; it also at the same time marks the start of the new research platform for One Health, which will be called the "One Health Platform".

The ongoing development of the national research platform, with the inclusion of the environmental sector and a further internationalisation of the activities, shows the basic holistic idea of the One Health approach in infection research.

You, esteemed colleagues, are the basis and the driver of this development. With your basic and applied research, you are contributing to promoting the understanding of the existence, diversity and infection dynamism of pathogens. The communication of your research results and the description of complex links supports the veterinary, health and environmental sector, for example in determining the risk factors for spillover infections and the epidemic potential of such infections

The dovetailing of the "4 C's" that are crucial to the One Health approach: collaboration, communication, coordination and capacity-building, is key for achieving the goal of sustainably balancing and optimising human, animal and environmental health.

(BMBF)

On 9 February, the Federal Ministry of Education and Research (BMBF) published the funding guidelines to promote the Research Platform for One Health. This inter-ministerial initiative is, as I already mentioned, no longer only supported by the previous four but by a total of six ministries. This step underlines the Federal Government's joint path towards One Health.

We are delighted that the two branches of the office of the zoonoses platform, the University of Münster and the Friedrich Loeffler Institute, will in future work together with the new partner, the newly established Helmholtz Institute for One Health in Greifswald (HIOH), in order to develop the research platform for zoonoses into a research platform for One Health.

As of December, the Federal Ministry of Education and Research will support the work of the three sites of the office for five years. The budget appropriation has already been granted (subject to update).

Tried and tested elements will be continued in the new research platform and new elements will be added: for instance, in future, funding will be provided for an annual One Health Symposium and other important networking formats, in particular for young scholars and scientists. It is also planned to provide funds for innovative research projects that adopt a One Health approach. A research agenda is being drawn up for this purpose. The new aspects will include greater links with graduate schools via a series of online seminars on One Health, in order to integrate One Health expertise into the education and training as early as possible, and the funding of international exchange, for instance via short-term research internships or workshops.

The new office will again be supported by a scientific advisory board, which will cover the range of specialist areas involved in the enhanced platform. All interested scientists from the One Health community (directed to the audience) - that is you, who constitute the foundation of this whole endeavour, are cordially invited to apply for a position in the advisory board.

The efforts to support One Health research are ongoing at European level and within the scope of global health. To this end, the BMBF is supporting the preparations for a new One Health AMR partnership set to begin in 2025. This initiative places a focus on antimicrobial resistances in pathogens present in humans, animals and the environment. This year also saw the launch of ADAPT, a new One Health network, as part of the Research Networks for Health Innovations in Sub-Saharan Africa. The aim of the

project is to work with governmental, local and regional actors in seven sub-Saharan African countries to build up capacities for improving the management of AMR and neglected tropical diseases and the handling of antimicrobials, employing a One Health approach.

(BMEL)

The Friedrich Loeffler Institute (FLI - Federal Research Institute for Animal Health), which is subordinate to the Federal Ministry of Food and Agriculture, started to establish two FLI base stations last year. Under the coordination of the Institute for International Animal Health / One Health, the aim is to create an infrastructure that, in addition to the flexible capacity building, also has a regional focus on cooperative cutting-edge research, education and training. In the middle of July, the cooperation agreement was signed with Zanzibar, Tanzania. Another binding agreement with Vietnam is planned. In future, One Health lighthouse projects will be initiated in cooperation with the FLI to provide the data required to provide scientifically-sound proof that One Health leads to measurable improvements in all three sectors. The focus is on the challenges facing our partner countries, because improving animal health and fighting zoonoses in the countries where they originate will also mean that fewer newly emerging pathogens reach Europe and Germany.

Highly Pathogenic Avian Influenza, via H5 viruses, is omnipresent this year, as it was last year, and the largest panzootic ever recorded is currently still highly dynamic. This not only has an impact on poultry and other kept birds but also has severe consequences for wild bird populations. Even if the zoonotic risk for these H5 viruses is currently still regarded as low, the increasing number of transmissions to carnivores such as foxes, minks, seals, sealions and cats calls for particular vigilance.

(BMG)

One Health research lives off interdisciplinary and transdisciplinary exchange between scientists from different specialist areas. Without this exchange, it is not possible to understand the complex interactions between human, animal and ecosystem health. At ministerial level as well, the importance of cross-ministerial cooperation in line with the One Health approach is growing continually, and results from cross-disciplinary research projects are often used.

The Federal Ministry of Health is currently involved in a number of such cross-ministerial projects. One prominent example is the surveillance of pathogens in waste water. The waste water-based surveillance of SARS-CoV-2 was initiated in several research projects, including with funds from the EU Commission and the Federal Ministry of Education and Research. The Federal Ministry of Health will now, in cooperation with the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, fund the establishment of permanent structures for the surveillance of waste water in Germany.

Another example is the German Antimicrobial Resistance Strategy; the Federal Ministry of Health recently coordinated a revision of the strategy, which has now been adopted by the Federal Government. The results from interdisciplinary research on antimicrobial resistance and on the use of antibiotics were able to provide important stimulus in this revision. Research in this area will also continue to play an important role in implementation.

(BMVg)

In some areas, health and safety are directly linked, and this is intensified by the effects of globalisation. The Ebola epidemic in western Africa and the COVID-19 pandemic have, for instance, shown the enormous influence of such infections on political, social and economic capacities. The potential weakening of State structures associated with this has enormous potential to destabilise. These global crises particularly underline the special role that armed forces play, over and beyond national and Alliance defence. Maintaining the operational capability of the German armed forces for the entire range of operations therefore requires the Federal Armed Forces Medical Corps to be able to react quickly to unexpected illnesses and events. In addition to this, the current wars and conflicts have shown the necessity for ongoing research in the area of medical ABC protection in order to also be able to react to the use of such weapons.

Last year, along with prevention activities, the Institute for Microbiology also initiated and expanded projects on treating infections with multi-resistant germs, for example through the use of novel active substances or specific phages, which may in future have sustainable success in fighting diseases.

In order to enable other nations to benefit from the Federal Armed Forces' findings and knowledge gained, a large-scale field exercise to identify and control a fictitious highly-pathogenic organism was successfully organised and monitored in Mauritania in cooperation with the countries of the G5 Sahel zone. Likewise, surveillance studies in animal populations in southern Tunisia were carried out with our partners on the ground, focusing on pathogens that represent an emerging burden for public health. For decades, the Federal Armed Forces have researched specific areas at home and abroad in a One Health approach and worked in practice in multiprofessional teams of medical professionals, pharmacists, veterinarians, scientists and social scientists. The goal is to develop solution-oriented knowledge management of all public health aspects, ranging from information gained using surveillance and med-

ical intelligence to create a digitalised analysis of the situation, via crisis management and communication to the necessary measures for health protection of the soldiers.

The forthcoming days of the symposium on the “Benefit and Chances of One Health Research” will offer you first-class sessions, discussions of new findings and developments, and the opportunity for an interdisciplinary and transdisciplinary exchange of thoughts and information.

So let us jointly ensure that One Health becomes one way, or rather the way, to live.

This only leaves me to wish us all a successful event, interesting presentations and inspiring discussions.

Thank you very much for your attention.

Federal Ministry of Education and Research (BMBF)

Federal Ministry of Health (BMG)

Federal Ministry of Food and Agriculture (BMEL)

Federal Ministry of Defence (BMVg)

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 185

Thirty invasive listeriosis outbreaks and ready-to-eat fish products

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Invasive listeriosis, caused by *Listeria (L.) monocytogenes*, is a severe foodborne infection with high mortality.

We used WGS and core genome multi locus sequence typing (cgMLST) for subtyping and cluster detection of *L. monocytogenes* isolates from listeriosis cases and food and environmental samples and conducted standardised patient interviews.

We identified 30 independent listeriosis outbreaks in Germany that are most likely associated with the consumption of smoked and graved fish products, with cases from 2010-2023, of which some are likely ongoing. In total, 367 outbreak cases were identified (2-50 cases per outbreak), 69 deaths reported and 21 of these died because of listeriosis. Many of these outbreaks were cross-border with further cases in EU member states. Most of the outbreaks were most likely associated with salmon products (293 cases), followed by trout (52 cases), halibut (16 cases), mackerel (4 cases) and matjes products (2 cases).

These data show that ready-to-eat fish products pose a serious risk for listeriosis infection. Therefore, food producers should improve industrial hygiene measures to minimise the risk of infection for consumers. In addition, vulnerable individuals (e.g. in hospitals, nursing homes) should not be catered with and better informed about ready-to-eat fish products and associated risks. To identify the source of these outbreaks, interdisciplinary One Health efforts, including WGS and epidemiological investigations, were essential.

Keywords:

Listeriosis, Outbreaks, WGS, One Health

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 227

Linking human and veterinary routine data on *Campylobacter* spp. - A One Health approach using secondary data

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The majority of reported human cases of Campylobacteriosis is foodborne. In the veterinary sector samples are taken based on statutory basis including specific projects aiming at consumer protection, e.g. the zoonoses monitoring. In parallel, data on foodborne diseases and outbreaks in humans are collected by the competent health Authorities. However, joint collection of data or analyses of these data are missing.

Within a One Health approach we first assess which routine data are available and which secondary data analyses are possible. Second, we jointly analysed *Campylobacter* spp. data of different collections from Lower Saxony for 2017-2020.

About 2.500 positive samples from the Lower Saxony State Office for Consumer Protection and Food Safety laboratory information system were included in the analyses. From the human sector, data on approx. 10.000 reported cases could be analysed. Data from the Animal Disease Reporting System and meteorological data were also included.

The different targets of the data collections in human and veterinary public services yield into differences in the type and depth of the information collected in both sectors. Thus direct linking of data is possible only to a limited extent. To draw more accurate conclusions, further information such as consumer behaviour is needed. However, a general increase in information may assumed if data is generally extended and a subselection of data is carried out in the course of a more focused use case.

Keywords:

foodborne infections, zoonosis, human health, animal health, integrated analysis

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 230

Development of forecasts and quality control charts for *Campylobacter* spp. cases in Lower Saxony, Germany using the One Health Approach

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With 60,000 – 70,000 reported cases/year (Robert Koch Institute), Campylobacteriosis is the most common notifiable bacterial disease in Germany. Poultry meat is considered the main source, but raw milk, raw meat, contaminated drinking water or contact with pets can also be a source of infections.

The aim of this study is to develop quality control charts as a benchmarking system using forecasts to detect deviations in the number of human *Campylobacter* spp. infections in Lower Saxony. The second aim is to assess whether integrating veterinary data regarding the One Health approach is possible.

Routine data from 2017 to 2020 provided by the Public Health Agency of Lower Saxony (human notified cases) and the Lower Saxony State Office for Consumer Protection and Food Safety (laboratory data of food samples) are analysed. Laboratory data include results from meat and meat products and milk and milk products. Models of the ARIMA family are used for forecasting. For quality control charts, the 95%-confidence interval of the forecast is used as the control limit.

Results show a better model for *Campylobacter* spp. cases without the One Health approach. In general, the forecast using the One Health approach estimates higher case counts compared to the forecast without it and the observed cases. The quality control charts show only a slight deviation of cases from the forecast in 2020, indicating an unexpected change in case counts.

Keywords:

One Health, *Campylobacter*, Forecast, ARIMA, Secondary Data

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 269

Horses as sentinels for the circulation of flaviviruses in eastern-central Germany

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Since 2018 autochthonous West Nile virus (WNV) infections have been regularly reported in eastern-central Germany. While clinical infections in humans and horses are rare, seroprevalence studies in horses may allow tracing virus transmission and consequently help to estimate the risk of human infections. In 2022, sera from 1232 unvaccinated horses were tested using a competitive pan-flavivirus ELISA (cELISA). Positive and borderline results were confirmed by virus neutralization test (VNT) against WNV, tick-borne encephalitis virus (TBEV), and Usutu virus (USUV). In addition, questionnaires were filled in for each horse in order to investigate possible risk factors for seropositivity against WNV and TBEV. These risk factors were calculated through logistic regression for WNV and via linear mixed model for TBEV.

Overall, 125 horse sera reacted in the cELISA, in the VNT, 114 could be differentiated and assigned to one of the flaviviruses while three showed antibodies against more than one virus, and eight couldn't be determined. The overall seroprevalence was 3.3% (95% CI: 2.38-4.40) for WNV and 5.60 % (95% CI: 4.44-7.04) for TBEV, and 0.41% (95% CI: 0.14-0.98) against USUV.

Risk factors for WNV seropositivity found in a previous study (previously registered WNV cases in the county & high estimated Mosquito numbers) could not be confirmed. Age was the only factor predicting TBEV seropositivity. We conclude, that horses are useful sentinels to determine flavivirus circulation.

Keywords:

vector-borne zoonoses, specific antibodies, risk factors, Usutu-, West Nile- and Tick-Borne Encephalitis-Virus

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 323

Seroprevalence of Crimean-Congo Hemorrhagic Fever in Dromedary Camels and Human Occupational Contacts in the United Arab Emirates

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Background: Crimean–Congo hemorrhagic fever (CCHF) has been reported in the United Arab Emirates (UAE) since 1980 and is considered as a public health threat in the country. This study was conducted to estimate the seroprevalence of CCHF in dromedary camels and abattoir workers in the UAE.

Methods: A cross-sectional study was conducted between March 2022 and June 2023 at the Al Bawadi abattoir in the Al Ain City on 393 camels and 86 abattoir workers. Anti- CCHFV IgG antibody was tested in the sera of camels using a multispecies indirect enzyme linked immunosorbent assay, ELISA. Furthermore, anti- CCHFV IgG antibody was tested in the sera of abattoir workers using the Human CCHFV ELISA Kit. Data were analyzed using SPSS version 28 (IBM Corporation).

Results: The seroprevalence of CCHF in dromedary camels was 65.1% (95% Confidence interval, CI = 0.604-0.699). No significant association was observed between the seroprevalence of CCHF and either sex ($\chi^2 = 1.04$, $p > 0.05$), age ($\chi^2 = 0.92$, $p > 0.05$), or body condition ($\chi^2 = 3.33$, $p > 0.05$) of the study camels. The seroprevalence of CCHF in abattoir workers was 27.9% (95% CI = 0.184-0.374).

Conclusion: The seroprevalence of CCHF was high in both camels and abattoir workers warranting for appropriate control and preventive measures.

Keywords:

Abattoir workers, Crimean-Congo hemorrhagic fever, Dromedary camels, Seroprevalence, United Arab Emirates

Session 1: Epidemiology and Secondary Data Use / Abstract-ID 215

Detection of pathogenic *Leptospira* spp. serogroups in Europe between 2017 and 2020 applying a gene-based molecular approach

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A wide spectrum of mammals, including dogs, can acquire leptospirosis resulting in the shedding of *Leptospira* spp. with urine. Canine vaccines in Europe contain two to four leptospiral serogroups. The lack of cross-protection among leptospiral serogroups makes the continuous evaluation of epidemiology necessary to assess the suitability of current vaccines and identify shortcomings to protect dogs.

Residual DNA from canine blood and urine ($n = 239$) was collected when *L. spp.* infection was suspected, and the *lipL32*-PCR displayed positive results. The remaining DNA was analyzed using a novel molecular serogroup typing consisting of a 16S rRNA endpoint PCR followed by 16S rRNA gene amplicon sequencing to identify the respective leptospiral genospecies. According to the species identified, a PCR with serogroup-specific primers for serogroups Icterohaemorrhagiae (ICT), Australis (AUS), Pomona (POM), Canicola (CAN), Autumnalis (AUT), and Pyrogenes (PYR) was performed.

The new PCR was able to type the leptospiral serogroup in 172 samples. The most prevalent *L. spp.*-serogroup identified in Europe was ICT (53%), followed by serogroups AUS (13%), POM (5%), and AUT (4%). Considering the occurrence of ICT, AUS, and GRI in rodents a core vaccination with these serovars is important, while the inclusion of the host-adapted serogroup CAN is recommended. This work shows that current L4 vaccines are relevant and should confer reliable protection against ICT and AUS serogroups.

Keywords:

Leptospira; Leptospirosis; Epidemiology; PCR; molecular serogroup typing; Canine leptospirosis

Session 2: Vaccines & Immunology / Abstract-ID 206

Exploring bat innate immune cell responses to filoviruses

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Bats are natural reservoirs of zoonotic viruses, including Marburg virus (MARV, naturally transmitted by Egyptian rousette bats; *Rousetus aegyptiacus*) and Bombali ebolavirus (BOMV, recently isolated from Angolan free-tailed bats; *Mops condylurus*). Their ability to host highly-pathogenic viruses likely correlates with co-evolved anti-viral immune response mechanisms. How bats achieve efficient innate or adaptive immune responses, without developing disease, remains to be addressed. Here, we profiled bat immune responses of dendritic cells (DC), key cellular targets of filoviruses. We infected DCs from rousette bats with recombinant MARV or Sudan ebolavirus (SUDV) expressing fluorescent proteins, allowing for a direct comparison between a filovirus these bats harbour naturally (MARV), and a filovirus efficiently cleared by rousette bats *in vivo* (SUDV). Despite similar viral titres, DCs supported increased intracellular MARV-ZsG replication by 3 days post-infection, while ZsG median fluorescence intensity (MFI) in SUDV-infected bmDCs decreased, indicating differential control of infection. MARV- and SUDV-infected DCs both upregulated CD40 and HLA-DR surface expression, indicating functional cell activation. Our findings highlight that despite being susceptible to infection, bat DCs display unimpaired antigen presentation capacities following filovirus infections, in contrast to the described impaired maturation and functionality observed in filovirus-infected human DCs.

Keywords:

bat, filovirus, dendritic cells, immune response

Session 2: Vaccines & Immunology / Abstract-ID 260

Toxoplasma gondii attenuated by low energy electron irradiation induces protective immune responses

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Vaccines against parasites must elicit immune responses against antigens present in different life cycle stages to provide sufficient immunity against infection. Attenuation of parasites is therefore a promising strategy, since they retain enough virulence to cause subclinical infection as well as metabolic activity to change their antigen composition.

Low energy electron irradiation (LEEI) is a novel approach for attenuating parasites. LEEI damages nucleic acids while antigen structures remain largely intact. LEEI offers advantages compared to other radiation technologies including precise dosing, reproducibility, a fast process and safety. It can be integrated into standard and GMP-compliant laboratories, requiring only minimal shielding constructions. We have developed a microfluidic-based LEEI process to attenuate *Toxoplasma gondii*, an important zoonotic parasite. *In vitro* analysis showed that the parasites could invade host cells but were impaired in their intracellular replication after LEEI. Suitable doses and process parameters for reproducible attenuation were identified. An immunization study in mice was conducted, comparing different LEEI-doses with chemically inactivated material. LEEI-attenuated tachyzoites elicited high levels of antibodies after immunization and protected the animals from acute infection. These results imply that LEEI is a valuable technology for attenuation of parasites and form the base for further development of anti-parasitic vaccines.

Keywords:

Toxoplasma gondii, Irradiation, Attenuation, Vaccine

Session 2: Vaccines & Immunology / Abstract-ID 257

Mice immunized with different recombinant West Nile virus proteins show varying levels of serological cross-reactivity and protection from infection

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West Nile Virus (WNV), a zoonotic flavivirus transmitted by mosquitoes, mainly causes moderate or no symptoms in infected individuals, however in some cases a neurological course of the infection can be severe or even fatal. To date, no human vaccine against WNV is available. The Envelope protein (E), located at the viral surface, mediates binding to and entry into host cells and is the major target for neutralizing antibodies and central to vaccine development. Flaviviruses share a high sequence homology, and conserved epitopes such as the fusion loop domain (FL) in the E, that can elicit cross-reactive antibodies. As a result, antibody dependent enhancement of infection is a major concern for vaccines in areas where multiple flaviviruses, such as dengue or Zika viruses, co-circulate. To reduce the potential of inducing cross-reactive antibodies, we performed an immunization study in mice using recombinantly expressed WNV Es with either wildtype (wt) sequence, or four point mutations in the FL, and WNV E domain III which lacks the FL. All antigens induced high levels of WNV-binding antibodies, but displayed varying protection efficiency. While the wt E conferred complete protection, the other antigens showed only partial protection. However, serological cross-reactivity to heterologous flaviviruses was significantly reduced with the mutated E. These results have indications for choosing antigens with optimal specificity and efficacy in WNV vaccine development.

Keywords:

West Nile Virus
Vaccine
recombinant Protein

Session 2: Vaccines & Immunology / Abstract-ID 184

Neutralization of the hepatitis E virus by porcine serum antibodies in a cell-culture based assay

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Wild boars and domestic pigs are the main reservoir hosts for the hepatitis E virus (HEV). Therefore, consumption of raw meat and other animal products pose a serious risk for human HEV infections. So far, the HEV prevalence in domestic pig and wild boar has been determined by RT-PCR and antibody ELISA. To characterize neutralizing properties of porcine anti-HEV serum antibodies, a neutralization assay was established using the human hepatoma cell line PLC/PRF/5, and the human genotype 3 HEV strain 47832c. Before application in the assay, it was imperative to treat the virus with bile acid to remove the quasi-envelope of the viral particles enabling binding of antibodies to the virus particle surface. After incubation of the virus with dilution series of the sera, the mixtures were added to the cells and the infection rate was evaluated one week later by immunofluorescence staining to calculate the neutralizing titer (ND50).

The antibody status of 343 wild boar sera collected in Lower Saxony, Germany, was determined by an in-house ELISA with a detection rate of 19 % anti-HEV IgG positive samples. A subset of 41 HEV RNA-negative and lowly to highly ELISA-reactive serum samples were investigated in parallel in the neutralization test. It could be shown that the ND50 correlates well with the corresponding s/p-ratio in ELISA. Neutralizing capacities of additional sera, which were positive for both, anti-HEV antibodies and HEV RNA, were also proven in the newly established assay.

Keywords:

hepatitis E virus, porcine antibodies, virus neutralization, assay development

Session 2: Vaccines & Immunology / Abstract-ID 244

Improving Clinical Trials for Candidate Vaccines Against AMR Infections: Perspectives from the COMBINE Project

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Background. Although preventive approaches are a promising tool to limit antimicrobial resistance (AMR), most pathogens on the WHO AMR priority list still lack a licensed vaccine. The feasibility of clinical studies to approve vaccines against (hospital-acquired and opportunistic) AMR pathogens is a major bottleneck. Hence, one goal of the COMBINE project, part of the IMI AMR Accelerator, is to improve the design of clinical trials to study the efficacy of candidate vaccines.

Methods. We have conducted a literature search and hosted a stakeholder workshop on recurrent problems in vaccine development. The results of these exercises are driving the re-analysis of individual patient data from past clinical trials as well as the examination of trial meta-data.

Results. Two major recurring problems in the clinical development were identified. The first issue is the lack of established correlates of protection, which makes it necessary to engage in large, resource-intensive clinical trials with prevention from the disease as primary endpoint. The second issue is the characterisation of the optimal target population - complicated, among others, by uncertainties around the risk factors.

Conclusions. The ultimate outcome of this work is to provide recommendations to facilitate the clinical development of candidate vaccines against AMR infections.

This work has received support from the EU/EFPIA Innovative Medicines Initiative 2 Joint Undertaking (COMBINE grant n° 853967).

Keywords:

Antimicrobial resistance; Vaccines; Clinical trial design; COMBINE

Session 2: Vaccines & Immunology / Abstract-ID 279

Consequences of *Ascaris*-*Salmonella* co-infection on immune functions in the pig

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Background

Infections with the parasitic roundworm *Ascaris suum* and the bacterial pathogen *Salmonella enterica* ssp. *enterica* ser. Typhimurium are widespread in pigs and both pathogens are highly prevalent zoonotic agents. Interestingly, there is a statistical association between high *Ascaris* exposure and *Salmonella* prevalence within a pig herd. The immune response against *A. suum* is characterized by a Th2 response whereas the control of *Salmonella* requires the development of an opposing Th1 immune response. An important interface between the two pathogens is represented by macrophages; helminth infections lead to alternative activation of macrophages with anti-inflammatory properties while *Salmonella* achieves persistence by surviving within macrophages with features of alternative activation.

Methods

To study phenotypic changes in macrophages during *Ascaris* infection and assess whether these changes promote *Salmonella* persistence within the porcine host, various organs from infected pigs were analyzed using flow cytometry. *Salmonella* burden was assessed by bacterial colony counting.

Results & Conclusion

Preliminary findings indicate that *Ascaris* infection is associated with a Th2-type response resulting in higher *Salmonella* burdens compared to pigs infected with *Salmonella* alone.

Keywords:

Ascaris, helminth, salmonella, coinfection, macrophage, immunity

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 277

Identification of possible peripheral routes for borna disease virus 1 (BoDV-1) infection

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BoDV-1 can cause severe encephalitis in humans but the transmission routes remain elusive. In a previous study, we demonstrated that BoDV-1 infection caused severe encephalitis in rhesus macaques following intracerebral inoculation. Furthermore, one third of BoDV-1 animals inoculated by a combination of peripheral routes had to be euthanized.

In a follow-up study we aimed to better define the peripheral route leading to disease manifestation.

Twelve adult rhesus macaques (*Macaca mulatta*) were inoculated with BoDV-1. Six animals were inoculated by the intranasal (i.n.) route and the other six were inoculated by the subcutaneous (s.c.) route; reflecting two possible entry sites. All animals were monitored for signs of disease and viral shedding. After four months all surviving animals were euthanized. Tissue material, cerebrospinal fluid and blood were analyzed for histopathology and viral load determination.

All six intranasally and three of six subcutaneously infected monkeys developed severe neurological signs. The i.n. inoculated animals developed symptoms after 4-6 weeks post-inoculation and disease duration was up to three weeks. The s.c. inoculated animals had a later onset of disease (10-12 weeks post-inoculation) and disease duration was up to two weeks.

Inoculation of rhesus macaques with BoDV-1 by both infection routes resulted in encephalitis. Although the disease is highly rare in humans, it is likely that humans may become infected through the same routes.

Keywords:

borna, encephalitis, animal model

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 290

Zoonotic potential of animal sarbecoviruses related to SARS-CoV-1 and SARS-CoV-2

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Animal sarbecoviruses closely related to SARS-CoV-1 and SARS-CoV-2 have been identified in bats and other animal species at the genomic level, however their zoonotic potential is unclear. Here, we used pseudoviruses bearing sarbecovirus spike (S) proteins to investigate species tropism, host cell factor usage, and inhibition by SARS-CoV-2-specific antibodies. Our findings reveal that S proteins from various bat and pangolin sarbecoviruses facilitate entry into human cells via the ACE2 receptor. In addition, two S proteins were found to enable ACE2-independent entry that required S protein activation by trypsin. Moreover, we observed that ACE2 orthologues from raccoon dog ACE2 could serve as an efficient sarbecovirus entry receptor. Finally, SARS-CoV-2 convalescent and vaccinated plasma displayed varying degrees of cross-neutralization against sarbecovirus S protein-bearing pseudoviruses, with plasma of quadruple vaccinated individuals showing high level of cross-neutralization.

Our study provides evidence that multiple animal sarbecoviruses can enter human cells without adaptation and highlights the potential role of raccoon dog as intermediate host. Further, we demonstrate that SARS-CoV-2-specific antibody responses may offer cross-protection against infection by certain animal sarbecoviruses. These findings contribute to a better understanding of the zoonotic potential of animal sarbecoviruses and have implications for public health interventions and surveillance strategies

Keywords:

SARS-CoV-1/ SARS-CoV-2 related coronaviruses, receptor, neutralization

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 246

In vivo models for rustrela virus (RusV) infection in potential reservoir and spill-over hosts

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Rustrela virus (RusV; species *Rubivirus strelense*) is a recently discovered relative of the human rubella virus and causes fatal non-suppurative meningoencephalomyelitis in various domestic, wild and zoo animal species in Germany, Austria and Sweden. Based on its reportedly broad range of susceptible hosts, a zoonotic potential of RusV cannot be excluded. Apparently healthy yellow-necked field mice (*Apodemus flavicollis*) and wood mice (*Apodemus sylvaticus*) were identified as potential reservoir hosts. Questions regarding the biology and epidemiology of RusV in reservoir and spill-over hosts remain elusive, such as course of infection, pathogenesis, and transmission routes. First attempts of virus isolation have failed and in vivo infection models have not been established.

Within the “RubiZoo” project, we therefore inoculated wood mice, representing potential reservoir hosts, and Lewis rats, potentially representing spill-over hosts, with brain homogenates originating from RusV-infected animals. Intracranial as well as combined intranasal/peroral inoculation resulted in infection with comparable kinetics in both species, whereas combined intramuscular/subcutaneous injection was less efficient. Viral loads were highest in the central nervous system but the virus spread also to peripheral organs, particularly adrenal gland and urinary bladder. Neither wood mice nor rats developed any clinical signs attributable to the infection until at least eight weeks post infection.

Keywords:

rustrela virus, animal models, reservoir hosts, spill-over hosts

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 276

Omicron subvariant BA.5 efficiently infects lung cells

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Zoonotic transmission of animal sarbecoviruses threatens public health, as evidenced by the SARS epidemic and the COVID-19 pandemic. The attenuated Omicron variant dominates the COVID-19 pandemic since winter 2021 and attenuation is believed to be at least partially due to inefficient infection of lung cells. Here, we investigated whether reduced capacity to spread in the lung has been preserved during evolution of Omicron subvariants. We report that the spike proteins of Omicron subvariants BA.4 and BA.5, which are identical at the amino acid level, show increased cleavage by host cell proteases and augmented capacity to drive cell-cell fusion. Furthermore, BA.4/BA.5 spike facilitated increased entry into Calu-3 lung cells and augmented entry was due to deletion of H69 and V70 but was not associated with altered TMPRSS2 usage. Furthermore, increased Calu-3 cell entry of pseudotypes bearing BA.5 spike translated into augmented Calu-3 cells infection by authentic BA.5 virus. Finally, BA.5 spread in the nasal epithelium of ferrets and in the lungs of mice with much higher efficiency than previously circulating Omicron subvariants. These results indicate that attenuation of the Omicron variant can at least by partially lost during evolution of subvariants.

Keywords:

SARS-CoV-2, spike, BA.5, lung

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 188

Less Pronounced Immunopathological Responses Following Oral Butyrate Treatment of *Campylobacter jejuni*-Infected Mice

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The food-borne pathogen *Campylobacter jejuni* causes campylobacteriosis which is the most frequently reported bacterial diarrheal disease in industrialized nations. Given that campylobacteriosis cases are rising globally and antibiotic treatment is not recommended, infected patients would substantially benefit from alternative therapies. The short-chain fatty acid butyrate is known for bactericidal and anti-inflammatory effects. This prompted us to investigate disease-alleviating properties of butyrate treatment in a preclinical murine campylobacteriosis model. Therefore, following commensal gut microbiota depletion IL-10^{-/-} mice were challenged with 10⁹ viable *C. jejuni* cells by oral gavage and treated with butyrate via the drinking water (22 g/L) starting on day 2 post-infection. As early as day 3 post-infection, butyrate reduced diarrheal severity and frequency in treated mice, whereas on day 6 post-infection, gastrointestinal *C. jejuni* burdens and the overall clinical outcomes were comparable in butyrate- and placebo-treated cohorts. Most importantly, butyrate treatment dampened intestinal pro-inflammatory immune responses given lower colonic numbers of apoptotic cells and neutrophils, less distinct TNF- α secretion in mesenteric lymph nodes and lower IL-6 and MCP-1 concentrations in the ileum. In conclusion, results of our preclinical intervention study provide evidence that butyrate represents a promising candidate molecule for the treatment of acute campylobacteriosis.

Keywords:

Campylobacter, treatment, preclinical study, butyrate, scfa

Session 3: Pathogenesis & Pathology of Zoonotic Infections / Abstract-ID 248

Using population dynamics and transposon-directed insertion site sequencing (TraDIS) to identify bacterial factors essential for the egress from the neonate epithelial cell

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Non-typhoidal *Salmonella* (NTS) are a global health problem in human and veterinary medicine. Low hygiene conditions and young age the risk for systemic dissemination. We applied our previously established neonatal mouse model to examine *S. Typhimurium* pathogenesis and systemic distribution. Within host cells, NTS typically reside in a specialized membrane-bound compartment, the *Salmonella* containing vacuole (SCV). To overcome the epithelial barrier, NTS use effector proteins encoded by *Salmonella* Pathogenicity Islands (SPIs). We recently demonstrated that SPI2 effector proteins play an important role in transmigration of the SCV across intestinal enterocytes. In the present study, our aim is to further identify essential genes for breaching the intestinal barrier, enterocyte egress and subsequent systemic spread. We created a mutant library with random Tn5 transposon integrations and will orally administer it to newborn mice (input pool). We will subsequently compare it to populations isolated from different tissues (output pool). The results of this study will contribute to a better comprehension of the process of egress and systemic spread and potentially assist in development of new treatment and prevention strategies for NTS infections in the newborn.

Keywords:

Salmonella Typhimurium, Tn5 library, TraDIS, Egress

Session 4: Host-pathogen interactions 1 / Abstract-ID 288

Cross-talk between glyphosate resistance, bacterial stress response and pathogenicity traits in *Salmonella enterica*

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Glyphosate, the world's most used herbicide, is a potent antimicrobial and a metal chelator that may select for resistance in animal pathogens. In our previous work, we obtained a *Salmonella enterica* serovar Typhimurium mutant resistant to a glyphosate-containing herbicide Roundup (Pöppe et al., 2020), which had a mutation in the bacterial stress response regulator *rpoS* that controls expression of various pathogenicity traits. Here, we attempted to investigate the molecular mechanisms and effects of glyphosate and glyphosate resistance on bacterial stress response and host-microbe interactions.

We found that Roundup readily induces starvation response by upregulating stringent response regulator *rpoS* and *dps*, involved in iron sequestration during the stationary phase. The Roundup-resistant mutant showed reduced motility, biofilm formation and iron accumulation. Co-culture with porcine intestinal epithelial cells showed a higher replication rate in the resistant mutant but no changes in cytotoxicity. A global quantitative proteomics study confirmed the constitutive downregulation of the pathogenicity-associated traits such as motility, biofilm formation and iron scavenging in the resistant mutant during co-culture.

These results confirm a cross-talk between glyphosate stress, stringent response and expression of pathogenicity traits that results in a trade-off between increased glyphosate resistance and decreased pathogenicity.

Keywords:

glyphosate, resistance, *Salmonella*, pathogenicity, bacterial stress response

Session 4: Host-pathogen interactions 1 / Abstract-ID 197

Cell-intrinsic genomic reassortment of pandemic H1N1 2009 and Eurasian avian-like swine influenza viruses results in potentially zoonotic variants

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Evolution of influenza A virus (IAV) in animal hosts and particularly reassortment between different strains are two key factors involved in the generation of pandemic viruses. Particularly the “Swine flu” (Pdm-2009) spread worldwide and was introduced into the European swine population where it started to generate a variety of reassortants of unknown zoonotic risk for humans.

We established conditions for single or co infection and passaging in a novel swine lung cell line for the strains Pdm 2009 and swH1N1. After passaging, we isolated strains from the 64^o viral generation and investigated their genome composition. Single infection and passaging led to new variants carrying distinct mutations that were partially also found in natural swine IAV isolates. Importantly, co infection and passaging led to adaptation and generation of reassortant viruses (87%) bearing distinct mutations and mainly carrying PB1-PA-NA segments from Pdm-2009 in the swH1N1-genomic background. Of note, some of the co-infection passaged IAVs may pose a potential zoonotic risk for humans, since they were able to efficiently infect genuine human lung explant tissue ex vivo.

We successfully established a new in vitro model to investigate IAV evolution and reassortment under the influence of swine lung cells. This model might be a useful tool to prospectively evaluate the compatibility of IAVs to generate reassortants, which might represent a threat to the human population.

Keywords:

Swine, Influenza Virus, Reassortment, Evolution

Session 4: Host-pathogen interactions 1 / Abstract-ID 223

Multivesicular bodies and the hepatitis E virus life cycle – more than just release?

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A peculiar feature of the hepatitis E virus (HEV) is its reliance on endosomes for its release. This route, mediated by MVBs, can be targeted by drugs, yet little is known about viral replication. The latter is mediated via the viral polyprotein pORF1 comprising seven domains. Neither its putative proteolytical processing nor its subcellular localization is fully clarified. Here, we aim to decipher the latter with respect to subcellular structures associated to the viral replicase. These may be targeted to counteract viral replication.

When expressed ectopically, pORF1 accumulated in vesicular structures within the endosomal system. The localization to CD63-positive structure was most pronounced and an association to the MVB-resident viral protein pORF3 was observed. Expression of the polyprotein's seven subdomains Met (methyltransferase), Y, PCP (papain-like cysteine-protease), HVR (hypervariable region), X, Hel (helicase) and RdRp (RNA-dependent RNA-polymerase) revealed that PCP is the only domain localizing like the full-length protein. A PCP-deficient pORF1 mutant lost its naïve association to MVBs. Strikingly, both pORF1 and PCP alone displayed release into the extracellular space via exosomes.

In summary, pORF1 localizes to MVBs in a PCP-dependent manner and is released via exosomes. This advances understanding of the viral life cycle leading to rational drug-design, as replication and release could be coupled, which may even indicate capsid-independent spread.

Keywords:

HEV, pORF1, multivesicular bodies, exosomes, replication

Session 4: Host-pathogen interactions 1 / Abstract-ID 242

Cryo-electron tomography of influenza A and Ebola virus cell infection

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Cryo-electron microscopy approaches provide critical tools to inform on virus structure and mechanistic understanding of infection for vaccine development and drug design and hence facilitate pandemic preparedness. We have established a cryo-electron tomography (cryo-ET) workflow which we applied to study SARS-CoV-2, influenza A virus (IAV) and Ebola virus (EBOV) infected cells at molecular resolution and close to native environment to shed light on replication machinery and assembly.

We investigated how the IAV ribonucleoprotein genome segments undergo selective clustering that leads to a 7+1 bundle incorporated into assembling virions at the plasma membrane, and the structure of EBOV inclusion bodies as replication organelles. We reveal that IAV hemagglutinin (HA) remodels membrane compartments that interact with vRNPs in a Rab11-dependent manner. Cluster analysis revealed that vRNPs cluster with increased local concentration both inside and around these compartments, suggesting that HA-remodelled organelles play a role as a vRNP sorting platform with an important role in segment reassortment during coinfection. We show that EBOV inclusion bodies contain condensed nucleocapsids that form parallel packets destined for delivery to the plasma membrane. Overall, cellular cryo-ET represents an important tool to study emerging viruses and its applications for structural analysis and biosafety considerations will be discussed.

Keywords:

influenza A virus, Ebola virus, structure, cryo-electron microscopy

Session 4: Host-pathogen interactions 1 / Abstract-ID 245

A study of the interaction of insect-specific viruses from Bunyavirales with the mosquito host and the mosquito-transmitted Rift Valley Fever Virus

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The order Bunyavirales includes many arboviruses as well as insect-specific viruses (ISVs). ISVs naturally infect mosquitoes but in contrast to arboviruses, they cannot infect vertebrates. ISVs have gained significant attention in recent years because of their ability to interfere with arbovirus infection and, thereby, their potential use in the prevention of disease spread by insects. However, little is known about ISVs, belonging to the bunyavirales, regarding the infectivity of mosquitoes and derived cells, as well as their ability to interfere with arboviruses, like Rift Valley fever virus (RVFV, Bunyavirales). RVFV is transmitted by various *Aedes* and *Culex* mosquitoes and thereby ISVs and RVFV may share the same mosquito vector.

In this study, we characterized different ISVs of the Bunyavirales (Badu virus, Herbert virus, Gouleako virus), regarding their growth kinetics in *Aedes* and *Culex*-derived cells, followed by their infection characteristics in mosquitoes. Herbert and Gouleako virus replicated to high titers at 3dpi in all tested mosquito cells. Besides, the ability of these ISVs to interfere with RVFV infection is investigated. Studying and understanding the interaction and interference of bunyavirales ISVs with arboviruses, specifically of the bunyavirales order, increase our understanding of ISV-mosquito-arbovirus interactions and their potential use to reduce the burden of mosquito-borne viruses.

Keywords:

Insect-specific viruses (ISVs), Rift Valley Fever Virus

Session 4: Host-pathogen interactions 1 / Abstract-ID 255

NETosis during Influenza: A potential risk factor for severe bacterial co-infection in pigs?

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Influenza-A-viruses (IAV) may cause flu affecting the respiratory tract of humans, poultry and pigs. Co-infections with pathogenic lung bacteria are common and contribute to the severity of disease progression. Neutrophils are recruited to the site of infection where they can release neutrophil extracellular traps (NETs) to counteract invading pathogens. NETs consist of a DNA backbone spiked with antimicrobial components. Degraded NETs contain growth factors that enhance the growth of *Pasteurellaceae* bacteria.

We aimed to investigate whether IAV induce NETs in neutrophils and thereby initiate the growth of pathogenic lung bacteria. Bronchoalveolar lavage fluids (BALF) from diseased pigs (IAV-positive and -negative) was biochemically and microbiologically characterized and their influence on bacteria, neutrophils and the host-pathogen interaction studied.

We detected vesicular NETs, a specific release of NETs by viable neutrophils, in BALF of IAV-positive pigs by electron microscopy and an increase in NET-markers as H3Cit. Our data indicate that *Pasteurellaceae*, as *Actinobacillus pleuropneumoniae*, receive a growth boost from IAV-positive BALF. Furthermore, IAV-positive BALF has an inhibiting effect on the respiratory burst of neutrophils.

In combination with the finding that neutrophils fail to kill *Pasteurellaceae*, we conclude that factors, such as NETs, released by neutrophils during an IAV infection in pigs, contribute to the origin of a bacterial co-infection.

Keywords:

Neutrophils, Innate Immunity, Influenza, Pigs, Pasteurellaceae

Get-Together & Poster Viewing / Abstract-ID 211

Imported food-products in Germany - a relevant source for PVL-positive *Staphylococcus aureus*?

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Panton-Valentine leukocidin (PVL) producing *Staphylococcus (S.) aureus* can cause recurrent, large skin abscesses, which can lead to severe human infections and stigmatization. Although food is not considered a relevant source for human *S. aureus* colonization, the potentially grave consequences of infection with PVL-positive *S. aureus* require monitoring of food for this kind of bacteria.

Since 2014, the German NRL for Coagulase-positive Staphylococci incl. *S. aureus* has screened *S. aureus* isolates in routinely submitted and research samples for the occurrence of the *lukS-PV* gene as PVL marker. All *lukS-PV* -positive isolates were further typed, characterized and sequenced allowing to decipher phylogenetic relationships.

In total, 31 *S. aureus* strains have been identified as *lukS-PV* -positive (30/4144 investigated Methicillin-resistant *S. aureus* (MRSA) strains and 1/681 investigated Methicillin-sensitive *S. aureus* (MSSA) strains). More than half of the strains originated from studies that targeted imported food products and insects intended for food production purposes.

Our results indicate that PVL-positive MSSA/MRSA strains occur relatively often in imported fish, seafood and beef in contrast to food / food-producing animals from Germany and could cause a public health threat as clonal lineages differ considerably from common livestock-associated MRSA strains in Germany.

Keywords:

MRSA, food, Panton-Valentine leukocidin, virulence

Get-Together & Poster Viewing / Abstract-ID 306

Mapping TBE – A Literature Research of TBE in Germany

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Background: Tick-borne encephalitis (TBE) is an arboviral infection of the central nervous system and is considered as an important zoonosis in an area ranging from Europe to various parts in Asia. In Germany, between 250 and 700 human cases are reported annually. As no causative treatment exists, the most critical preventive measure is vaccination, but awareness among HCPs as well as lay population is relatively low and vaccination rates even in risk areas remain improvable.

Methods: A literature review was performed to identify locations of TBE-Virus (TBEV) natural foci, virus detection in ticks, as well as TBEV antibody detection in sentinel animals. Locations, including exact GPS coordinates where available, were mapped and overlaid with maps of TBE risk areas and areas where autochthonous human cases have occurred.

Results: Based on the literature research, separate maps for TBEV detection in ticks and seroprevalence in sentinel animals including foxes, sheep, goats, and horses revealed a concentration of potential natural TBEV foci in southern Germany but also throughout the entire country.

Conclusion: The maps demonstrate that TBEV-infected ticks and TBEV antibodies in sentinel animals are found nationwide in Germany. Hence, the risk of human TBE infection not only exists in official designated risk areas based on reported TBE incidence but throughout the country. This should be properly communicated and recommendations for vaccination should be extended accordingly.

Keywords:

tick-borne encephalitis, natural foci, seroprevalence, sentinel animals, map, Germany



Get-Together & Poster Viewing / Abstract-ID 330

UnCoVar: A Reproducible and Scalable Workflow for Transparent and Robust SARS-CoV-2 Variant Calling and Lineage Assignment

Authors: Alexander Thomas^{None}; Thomas Battenfeld; Olympia Anastasiou; Ulf Dittmer; Carina Eisner; Ivana Kraiselburd; Johannes Köster; Vu Thuy Khanh Le-Trilling; Simon Magin; Folker Meyer; René Scholtysik; Mirko Trilling; Pelin Yilmaz

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Genome sequencing became an indispensable tool to characterize the ongoing COVID-19 pandemic. We present a benchmark data set of 54 patient samples, sequenced with several different in-vitro approaches on two sequencing platforms, a comprehensive benchmark of existing workflows and introduce UnCoVar, an open-source bioinformatics workflow for analyzing SARS-CoV-2 sequencing data for patient samples and environments. The fully automated workflow assembles SARS-CoV-2 genomes, identifies lineages and offers high resolution variant calling. The workflow also includes multiple analysis approaches suitable for assembled genomes, variant-based consensus genomes and unassembled reads. UnCoVar includes extensive quality control and automated generation of a comprehensive report, that provides valuable insights for data consumers, for both researchers and clinicians. The software provides a configurable, user-friendly, scalable, and reproducible pipeline for SARS-CoV-2 genome sequence data analysis. It is implemented with Snakemake and Python and a containerized version is available. Redundant analysis paths of the workflow ensure robust results, producing submission-ready high-quality SARS-CoV-2 genome sequences, enabling molecular surveillance of the pandemic. The open-source code is available under a BSD 2-clause license at github.com/IKIM-Essen/uncover.

Keywords:

SARS-CoV-2, Workflow, Variant Calling, Lineage Assignment, Next Generation Sequencing

Get-Together & Poster Viewing / Abstract-ID 333

Detection of Bartonella quintana in lice collected from cloth of Ethiopian homeless

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Human lice *Pediculus humanus* can transmit various pathogens comprising *Bartonella quintana*, *Borrelia recurrentis* and *Rickettsia prowazekii*. Xenosurveillance is an epidemiological approach of assessing human infection risks by screening vectors of infectious diseases. In the proof-of-principle study conducted here, 23 human lice collected from the cloths of 30 homeless Ethiopian individuals were assessed by 16S rRNA gene-specific panbacterial PCR and PCR with specificity for relapsing fever-associated *Borrelia* spp. with subsequent sequencing of the amplicons. In one amplicon of the panbacterial PCR (4.3% of the assessed lice), DNA of *Bartonella quintana* was identified. Correlating clinical data were not available, however, the assessment confirmed the abundance of *B. quintana* in local lice and thus an associated infection pressure. Larger-sized cross-sectional studies seem advisable to more reliably quantify the infection risk for lice-infested local individuals. The need for prevention by providing options of maintaining standard hygiene for Ethiopian homeless individuals is stressed by the finding.

Keywords:

Ethiopia; xenosurveillance; *Pediculus humanus*; *Bartonella quintana*; infection risk; vector



Get-Together & Poster Viewing / Abstract-ID 195

Neuropathogenicity of an atypical anthrax causing bacterium – *Bacillus cereus* biovar anthracis

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Bacillus anthracis is capable of invading the central nervous system in both animals and humans. It can elicit meningitis, a severe complication of systemic anthrax, with a mortality rate nearing 100 %. In the past two decades, it has become evident that anthrax can be caused by bacteria distinct from *B. anthracis*. One such bacterium is *Bacillus cereus* biovar *anthracis* (*Bcbva*), which is responsible for wildlife deaths across sub-Saharan African rainforests. Among its hosts is one of our closest relatives, the chimpanzee, suggesting *Bcbva*'s zoonotic character. In this study, we investigated the previously uncharacterized neuropathogenic potential of *Bcbva*. We examined four formalin-fixed brains from chimpanzees that succumbed to *Bcbva* infections, using MRI and histology techniques, which were collected under strict biosafety measures as part of an ongoing wildlife health monitoring program in the Taï NP. Our findings revealed that, similar to *B. anthracis*, *Bcbva* is capable of breaching the blood-cerebrospinal fluid barrier and invading the meninges. Additionally, all cases exhibited *Bcbva* infiltration within the brain parenchyma, indicating a higher propensity to penetrate the glia limitans compared to *B. anthracis*. Moreover, *Bcbva* was found to extensively degrade brain tissue, as evidenced by significant extracellular matrix degradation. Limited activation of glial cells suggests a rapid demise following infiltration of the central nervous system.

Keywords:

Bacillus cereus biovar anthracis, Neuropathology



Get-Together & Poster Viewing / Abstract-ID 204

Inhibition of p38 MAPK during hyperinflammatory virus infections – achievements and challenges *in vivo*

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Zoonotic respiratory viruses crossing the species barrier to humans like SARS-CoV-2 or highly pathogenic avian influenza A virus (HPAIV) often lead to a rapid clinical deterioration in late disease stages, correlating with systemic hyperinflammation and requiring new immunomodulatory therapeutic approaches. We suggest that the key inflammatory MAPK p38 substantially drives the development of this immune dysregulation. Using clinically evaluated p38 inhibitors we significantly reduced the pro-inflammatory cytokine expression during SARS-CoV-2 infection *in vitro* in human lung cells and organoids as well as *ex vivo* in lung explants while maintaining the interferon-mediated antiviral response. Strikingly, we discovered a strong drug synergy between p38 inhibition and the antiviral remdesivir.

Despite of strong efficacy *in vitro* and *ex vivo*, daily application of p38 inhibitors in BALB/c and C57BL/6 mice provided controversial results. On the one hand, treatment from 72 h post infection led to exacerbation of low pathogenic influenza A virus infection. On the other hand, survival as well as pathogenesis during SARS-CoV-2 or HPAIV infection were not significantly affected by treatment from day of infection.

This work discusses p38 inhibition as promising therapeutic approach for hyperinflammatory virus infections and how to overcome the investigatory hurdle from *in vitro/ ex vivo* drug applications to *in vivo*, considering mouse models, drug applications and solvents.

Keywords:

Zoonotic viruses, SARS-CoV-2, Influenza A Virus, inflammation, treatment approaches, p38 MAPK

Get-Together & Poster Viewing / Abstract-ID 209

CRISPRi based gene silencing for the analysis of *Burkholderia* virulence factors in cellular infection assays

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The genus *Burkholderia* contains in part human-pathogenic species. Among them, the zoonotic disease-causing *Burkholderia pseudomallei*, *B. mallei*, and the avirulent *B. thailandensis* are genetically closely related. The intracellular lifestyle of these *Burkholderia* species involves different virulence factors which enable host cell invasion, phagosome escape, and actin-based intracellular motility. For the validation of CRISPRi based gene silencing experiments, BipC, a type 3 secretion system translocon, and the trimeric auto-transporter protein BimA, which is essential for actin-dependent intracellular motility of pathogenic *B. pseudomallei* group members. Here, we present results of cellular infection assays using different *B. thailandensis* gene knock-down strains used to further elucidate critical bacterial factors determining *Burkholderia* virulence.

Keywords:

Burkholderia, CRISPRi, gene silencing, virulence



Get-Together & Poster Viewing / Abstract-ID 214

In vivo determination of pathophysiological oxygen levels in ferret lung tissue during SARS-CoV-2 infection

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SARS-CoV-2 causes COVID-19 with varying disease manifestations ranging from asymptomatic to severe symptoms. Especially age is one of the main factors for people at risk. The SARS-CoV-2 virus primarily attacks pulmonary tissues and impairs gas exchange leading to acute respiratory distress syndrome (ARDS) associated with systemic hypoxia. Importantly, the level of tissue oxygen level affects the host cell behaviour that may initiate protective responses or cause detrimental consequences.

Our goal is to understand the processes of hypoxia in the elderly using aged ferrets as model. For that purpose, we infected nine aged (3 years-old) ferrets with SARS-CoV-2 and characterized clinical signs, viral load and the available oxygen in lung tissue at different days post infection (dpi). Oxygen levels were measured invasively in the tissue of anaesthetised and room air breathing ferrets using luminescence-based sensors.

The aged infected ferrets showed clinical signs accompanied with viral shedding. At 4 dpi lung and airway lesion scores were higher in infected than in uninfected ferrets, and at the same time, a decrease in tissue oxygen levels to 3,89 %O₂ was measured in infected lung tissue.

In conclusion, we successfully determined pathophysiological tissue oxygen level in lungs of aged SARS-CoV-2-infected ferrets. The data of oxygen levels obtained in vivo will be used to establish optimized in vitro methods that mimic hypoxia to study host cell responses during infection.

Keywords:

oxygen measurement, in vivo, SARS-CoV-2, COVID-19, animal model, lung tissue oxygen, ferret, aging



Get-Together & Poster Viewing / Abstract-ID 231

Antimicrobial profiles, pathogenic potential, and phylogenetic analysis of *Escherichia coli* isolated from slaughterhouses in Benin- City, Nigeria

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An important transmission route for foodborne pathogens is the contact of processed meat with contaminated surfaces. In our study, *E. coli* isolates from slaughterhouses in Benin City, Nigeria were characterized for biocide and antimicrobial susceptibility, biofilm formation capability, curli fimbriae and cellulose expression. In addition, whole genome sequencing (WGS) was performed to analyse the genetic diversity of the *E. coli* strains and to unravel the resistome and virulome of each isolate. Biocide susceptibility from our study population did not portray resistance to disinfectants since MIC and MBC values were well below in-use concentrations. 61% of the isolates formed biofilms while 31% produced curli fimbriae and/or cellulose. WGS analysis revealed a diverse phylogenetic architecture of the *E. coli* population. Among others, we identified enteropathogenic *E. coli* as well as isolates belonging to major sequence types of extraintestinal pathogenic lineages. Extended-spectrum β -lactamase (ESBL-) producing *E. coli* (n=2) were positive for blaCTX-M-15. Isolates carried plasmids responsible for biofilm formation and virulence promotion. Overall, data from our study revealed that meat processing environments can be a reservoir of ESBL-producing and colistin resistant *E. coli*, which could be culpable in the dissemination of pathogenic clones of environmental and public health concern.

Keywords:

Biocides, Antibiotics, resistome, biofilms, environmental health

Get-Together & Poster Viewing / Abstract-ID 286

PCMV glycoprotein B epitope mapping for a peptide-based detection of PCMV directed antibodies in pig

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Xenotransplantation of pig organ, tissue or cells depends on the microbial safety. Here the early detection of porcine cytomegalovirus (PCMV) a HHV6 related roseolovirus is immanent as it is easily transmitted and does possibly contribute to early graft failure. PCMV detection faces two hurdles. One is latency of the virus, the other high transmissibility and infectivity within the pig population. Even if PCMV free piglets are feasible, the avoidance of re-entry into a designated pathogen free (DPF) facility remains a challenge.

PCMV infection shows mild symptoms, furthermore the presence of maternal antibodies in newborn may cover a newly infection and virus latency impedes its detection. As such the screening is essential. It was recently shown that a combination of a PCR based testing combined with serology framed in a defined timely schedule is the gold standard.

By extending this approach, we analysed glycoprotein B (gB) for antibody binding epitopes on basis of a new peptide array representing 212 different 15 aa long overlapping peptides that cover the entire gB. The arrays were incubated with sera from non-infected and infected pigs identifying a variation of corresponding motives used for the synthesis of 25 aa biotinylated peptides tested in a diagnostic ELISA for suitability, monitoring pig anti-PCMV gB directed IgG and IgM antibody levels. It showed to be a suitable approach to complement the existing methods for PCMV monitoring of live-stock animals.

Keywords:

porcine cytomegalovirus, PCMV, roseolovirus, xenotransplantation, peptide array

Get-Together & Poster Viewing / Abstract-ID 213

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

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South American camelids (SAC) are popular in Europe, frequently kept with other livestock species and in close contact with humans. They represent a potential transmission source of epizootic and zoonotic bacteria to livestock and humans. Therefore, SAC were included as livestock species in the revised European Animal Health Law. However, knowledge on bacterial pathogens in SAC is too sparse for drafting appropriate monitoring and preventive medicine programs. To investigate the presence of Shiga toxin-encoding *Escherichia coli* (STEC) in SAC, 20 animals each were sampled at two different time-points in ten and then nine flocks. The herd prevalence determined was 70% in the first sampling round and 100% in the second. A total of 362 samples from individual animals were tested for stx-gene presence and 21 samples (5.8%) were PCR-positive for stx1, 51 for stx2 (14.1%) and 23 for both genes (6.4%). The intra-flock prevalence fluctuated widely, ranging from 0-53% in the first round and from 6-60% in the second round. The stx-gene subtypes identified were predominantly stx1c (98%; 41/42) and stx2b (63%; 50/80), which are considered as being of low risk for causing severe human disease. A few animals were also PCR-positive for stx1a, stx2c, stx2d, stx2e and stx2f. Multiple stx2 subtype signals were detected in 24% of the samples (19/80). In six stx2-positive samples, a subtype could not be assigned. Current efforts focus on identifying this stx2 subtype and on obtaining isolates.

Keywords:

Escherichia coli

Shiga toxin

South American Camelids

Prevalence

Get-Together & Poster Viewing / Abstract-ID 262

Panton-Valentine leukocidin (PVL)-producing *S. aureus* in a cat and its human owners – A case report

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Panton-Valentine leukocidin (PVL)-producing *Staphylococcus aureus* (PVL-SA) causes severe skin abscesses in humans. We report on an affected family experiencing recurrent PVL-SA infections despite multiple decolonization events. The family-cats were found to be colonized by *S. aureus* (SA). A protocol for outpatient decolonization using systemic antibiotic treatment of cats was developed.

Bacteriological investigation of samples from both cats revealed methicillin-susceptible SA in the oral cavity and nose of both cats. PCR testing for the presence of *lukF-lukS* identified a PVL-SA in one cat, while the second cat carried a PVL-negative SA. Comparative analysis of human and feline SA, performed by whole genome sequencing, demonstrated close clonal relationships of both the PVL-SA (ST8) and the PVL-negative SA (ST45).

Results of AST were used to develop a decolonization protocol based on oral therapy with amoxicillin-clavulanate for 10 d and 20 d, which resulted in a significant reduction of SA. The samples of the SA-carrying cat were negative after 10 days, but the PVL-SA-positive cat required a second course of antibiotics. Control examinations after 3 and 7 weeks were negative for SA.

The decolonization of cats was achieved by systemic antibiotic therapy and hygiene measures. The close relationship of human and feline isolates suggests transmission events in the household and underscores the importance of potentially colonized pets for the success of decolonization measures.

Keywords:

Panton-Valentine leukocidin (PVL)-producing *Staphylococcus aureus*, cat, decolonization, whole genome sequencing

Get-Together & Poster Viewing / Abstract-ID 273

Nasal *Staphylococcus aureus* colonization of horses and associated humans in the community and the question of mutual trans- mission

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Methicillin resistant *S. aureus* (MRSA) is nosocomial pathogen in horse clinics where it also frequently colonizes veterinary staff. Therefore, it was of interest to know about its probable spread of MRSA to healthy horses, into community and transmission to associated humans. The study reported here was based on samples from 254 horses and 156 humans in contact with them in 103 equestrian facilities located in 6 German federal countries. Overall rates of nasal *S. aureus* colonization were 6,3% (4% MRSA) among horses and 37,5% (0,64% MRSA) among humans. The MRSA isolates from horses as well as those from humans were attributed to the horse clinic associated subpopulation of LA-MRSA CC398. For these MRSA and also for methicillin susceptible isolates (MSSA), typing suggested transmission between horses and humans. Interestingly, particular clonal lineages among MSSA (ST816, ST1640 und ST1660) from horses seem to be specifically associated with this host¹. They are not registered in the database of the German National Reference Centre for Staphylococci and Enterococci among isolates from humans and other animal species during the past 30 years. In conclusion, our results confirm previous observations that nasal colonization with *S. aureus* in horses is infrequent². Introduction of horse clinic associated MRSA into the community is obviously rare.

¹ Kaiser-Thom S, et al., BMC Vet Res. 2022; 18: 79.

² Mama OM et al. Animals (Basel). 2019 Nov 1;9(11):900.

Keywords:

MRSA, transmission, nosocomial pathogen in horse clinics, zoonosis



Get-Together & Poster Viewing / Abstract-ID 177

Kidney Involvement in Schistosomiasis

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Schistosomiasis (Bilharziasis) is a Neglected Tropical Disease (NTD), which is caused by trematodes (flukes). One of the main species causing urogenital Schistosomiasis in humans is *Schistosoma haematobium*, which is highly endemic in sub-Saharan Africa.

S. haematobium has a complex life cycle. Humans are the final hosts and freshwater snails are the intermediate hosts. After a prepatent period, paired adult worms produce large amounts of eggs in the venous plexus of the bladder, which are shed from infected humans. However, some eggs trap in the tissue, leading to granulomatous formations and inflammation in urinary tract.

Here, we aim to investigate immune cells and Tubular Epithelial Cells (TECs) in urine as well as inflammatory and immune markers such as cytokines and antibodies against *S. haematobium* in blood samples. The hypothesis is: The more advanced the infection, the more immune cells, TECs and inflammatory markers are present in urine and blood.

Keywords:

Schistosomiasis, Trematodes, *S. haematobium*, Eggs, Inflammation, Bladder, Kidney, Immune cells, Tubular Epithelial Cells



Get-Together & Poster Viewing / Abstract-ID 180

Go With the Flow: *Streptococcus canis* Affects Endothelial Cell Migration in the Microfluidic Circulation

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Streptococcus canis (*S. canis*) is known as opportunistic pathogen colonizing dogs and cats, but also causes zoonotic diseases such as endocardites and septicaemiae in humans.

In the course of infection, *S. canis* enters the bloodstream and adheres to the endocardium as well as to the vasculature thereby inducing cell damage.

We aim to determine the impact of *S. canis* in particular on endothelial wound healing during cell culture infection. We used our established cell culture technique, which enables live cell imaging of the wound healing process after infection with streptococci. Additionally, differential immunofluorescence staining followed by confocal laser scanning microscopy was performed. Interestingly, incubation of endothelial cells (HUVEC) with *S. canis* causes cell damage and significantly inhibited endothelial gap closure.

With the aim to analyse the effect of *S. canis* infection on endothelial wound healing under physiological flow conditions present in the blood circulation, we combined the CSMA with a microfluidic system, which enables the application of defined shear stress values. Equally to infection under static conditions, circulating *S. canis* significantly inhibited endothelial gap closure at a defined shear stress.

The developed technique in combination with the microfluidic pump system proved to be ideally suited for the analysis of *S. canis* infection in the vascular system simulating systematic disease progression *in vitro*.

Keywords:

Streptococcus canis, endothelium, cell migration, microfluidic, wound healing



Human microbiota-associated IL-10^{-/-} mice: a valuable enterocolitis model to dissect the interactions of *Campylobacter jejuni* with host immunity and gut microbiota

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Secondary abiotic (SAB) IL-10^{-/-} mice constitute a valuable *Campylobacter jejuni*-induced enterocolitis model. Given that the host-specific gut microbiota plays a key role in susceptibility of the vertebrate host towards or resistance against enteropathogenic infection, we surveyed immunopathological sequelae of *C. jejuni* infection in human microbiota-associated (hma) and SAB IL-10^{-/-} mice. Following oral challenge, *C. jejuni* readily colonized the gastrointestinal tract of hma and SAB mice, but with lower numbers in the former versus the latter. Whereas hma mice were clinically less severely compromised, both, macroscopic and microscopic inflammatory sequelae of *C. jejuni* infection including histopathological and apoptotic cell responses in the colon of IL-10^{-/-} mice were comparably pronounced in the presence and absence of a human gut microbiota at day 6 post-infection. Furthermore, *C. jejuni* infection of hma and SAB mice resulted in similarly enhanced immune cell responses in the colon and in differential pro-inflammatory mediator secretion in the intestinal tract which also held true for extra-intestinal including systemic compartments. Notably, *C. jejuni* infection of hma mice was associated with distinct gut microbiota shifts. In conclusion, hma IL-10^{-/-} mice represent a reliable *C. jejuni*-induced enterocolitis model to dissect the interactions of the enteropathogen, vertebrate host immunity and human gut microbiota.

Keywords:

Campylobacter jejuni, enteropathogenic infection, acute campylobacteriosis model, microbiota-depleted mice, secondary abiotic IL-10^{-/-} mice, human gut microbiota associated IL-10^{-/-} mice, host pathogen interaction, gut microbiota shifts



Get-Together & Poster Viewing / Abstract-ID 194

Shifting towards the small cell variant form allows intracellular *C. burnetii* to withstand adverse microenvironmental conditions

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Coxiella burnetii is the causative agent of the zoonotic disease Q fever. Aside from acute fever, few patients can develop chronic Q fever months or years after primary infection, mainly characterized as endocarditis. The clinical picture of chronic Q fever suggests that *C. burnetii* establishes a persistent state. Yet, information about the induction of persistence is rare. STAT3 is important for host immunity and controls the expression of citrate transporter and citrate synthase. Under hypoxic conditions, stabilization of HIF1 α impairs the STAT3 activity, resulting in reduction of the TCA cycle intermediate, citrate. Citrate limitation results in inhibition of *C. burnetii* replication without interfering with the viability of the pathogen. This suggests that under hypoxia, *C. burnetii* might undergo stringent response or enters the metabolically inactive small cell-variant form (SCV) to survive nutritional limitation. To characterize *C. burnetii* under these conditions we infected primary murine macrophages with *C. burnetii* under normoxic and hypoxic conditions and analyse the expression of stringent response and SCV genes. Our data suggests that *C. burnetii* does not undergo stringent response, but instead enters the SCV as non-replicating persistent form. Further research is required to validate this assumption. For this bacterial morphology, bacterial ability to invade new target cells and to withstand adverse conditions, e.g. drug sensitivity will be analysed.

Keywords:

C. burnetii, hypoxia, small cell variant and persistence



Get-Together & Poster Viewing / Abstract-ID 201

The role of TMPRSS2 and other proteases in activation of influenza A virus in avian hosts

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Wild birds, primarily waterfowl, and shorebirds are the natural host and reservoir for influenza A viruses (IAV). Infections with low pathogenic avian IAV (LPIAV) in wild birds are usually asymptomatic, with virus replication primarily in intestinal enterocytes and significant virus shedding in faeces. Transmission to other hosts causes high morbidity and low mortality. Proteases that cleave HA, the prime determinant of avian IAV pathogenicity in poultry, are yet unknown but are believed to be restricted to avian respiratory and intestinal tissues. We previously confirmed in vitro that the transmembrane serine protease 2 (TMPRSS2) cleaves IAV and influenza B virus (IBV) HA with a monobasic cleavage site. Later studies revealed that TMPRSS2 is vital for proteolytic activation of almost all IAV HA subtypes in murine and human airway cells and for IBV in human lung. We now aim to elucidate its role in HA activation of IAV with monobasic cleavage site in avian species. TMPRSS2 from small intestine and lung of adult chicken and duck, respectively were able to support proteolytic activation and multicycle replication of IAV of different HA subtype in transient protease expressing MDCK cells, indicating that TMPRSS2 provides a promising HA activating candidate. Ongoing studies aim to reveal mRNA tissue distributions of TMPRSS2- and other proteases via RT-qPCR and comprehensive transcriptome analyses are performed to characterize the virus-activating protease repertoire.

Keywords:

Avian Influenza, Host-Proteases, Tropism, TMPRSS2



Get-Together & Poster Viewing / Abstract-ID 210

Impact of the M-like protein of *Streptococcus equi* ssp. *zooepidemicus* on the adherence to cardiac endothelial cells

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*Streptococcus equi*ssp.*zooepidemicus* (SEZ) is a zoonotic pathogen that colonises as commensal the equine tonsils and can cause respiratory tract infections in horses. It is also associated with endocarditis, meningitis, and severe systemic diseases in humans. When SEZ strain MF1397 was cultivated on solid media, colonies showed apparent differences in colony morphology and appeared both, mucoid and non-mucoid. According to genome sequencing data, several M- and M-like proteins are encoded in the genome of SEZ. A genome comparison indicates a frameshift within the gene sequence encoding the M-like protein. This leads to transcriptional termination and loss of the M-like protein.

M- and M-like proteins have already been characterised as important virulence factors in some streptococcal species such as *S. pyogenes* and they might be important for bacterial adherence to eukaryotic cell surfaces. Functional cell culture infection studies of both phenotypes to human primary endothelial cells revealed that adherence of the non-mucoid phenotype was significantly higher than adherence of the mucoid phenotype.

In sum, our data provide first evidence for the contribution of the surface appearance of the M-like protein to the observed culture heterogeneity and point to a significant implication for the bacterial adherence to host cells.

Thus, the occurrence of the phenotypes could directly play a crucial role in the efficiency of SEZ colonisation in the host environment.

Keywords:

Streptococcus equi ssp. *zooepidemicus*, M-like Protein, cardiac endothelial cells



Lack of potent inflammasome activation might prevent efficient *C. burnetii* clearance

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C. burnetii is a gram-negative, obligate intracellular bacterium and the causative agent of the disease Q fever. Its primary reservoir are ruminants. Human infection can occur via the inhalation of contaminated aerosols. After infection, Q fever often is either asymptomatic or manifests as a mild flu-like illness, but pneumonia or hepatitis might also occur. In the majority of cases, the bacteria are cleared, but patients can develop chronic Q fever even years after primary infection. This indicates that in these cases the host is unable to eliminate the pathogen. An inflammatory response would be required to facilitate the clearance of the bacteria. Inflammasomes are multimeric protein complexes that induce a pro-inflammatory response to combat pathogens. Here we show that *C. burnetii* fails to cause a strong activation of the NLRP3 inflammasome, which might result in a lack of bacterial elimination. Indeed, additional stimulation with an inflammasome activator leads to inflammasome activation and, as a consequence, to reduced bacterial burden. As bacterial infections induce low oxygen levels in the affected tissue, we also investigated the impact of different oxygen concentrations on *C. burnetii* infection. Hypoxia prevents *C. burnetii* replication, without bacterial clearance. The lack of bacterial elimination might be due to the lack of inflammasome activation. Thus, forced activation of the inflammasome might be a therapeutic option for clearance of *C. burnetii*.

Keywords:

NLRP3 inflammasome, inflammatory response, *C. burnetii*, hypoxia

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Salmonella Pathogenicity Island 2 (SPI2) effectors facilitate enterocyte transmigration in neonatal non-typhoidal Salmonella infections

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Non-typhoidal *Salmonella* (NTS) belong to the most prevalent causes of infectious diarrheal disease in humans and pigs worldwide, but also contribute to invasive infections in infants. The pathogenicity of NTS is conferred by horizontally acquired chromosomal regions, called *Salmonella* pathogenicity islands (SPIs), encoding sets of effector proteins delivered into the host cell via specific type-three secretion systems. Several *in vitro* studies identified SPI2 as a requirement for the establishment of an intracellular compartment allowing bacterial survival and replication inside the host cell, the *Salmonella* containing vacuole (SCV).

We used our previously established neonatal mouse model to clarify the role of SPI2 in establishment and progression of systemic NTS infections in the neonate host.

Oral infection with wildtype and SPI2-deficient NTS resulted in similar bacterial loads of the gastrointestinal tract, whereas re-isolation rates of mutants from systemic organs were significantly decreased. In contrast to the general understanding of SPI2 as prerequisite for SCV formation *in vitro*, mutants established and maintained SCVs and even grew to high numbers without harming the host cell. By evaluating isogenic SPI2 effector protein deficient *Salmonella* strains, we demonstrate that the effector SifA significantly contributes to the SPI2-dependent phenotype *in vivo*. Its absence prevents transmigration of enterocytes and subsequent systemic dissemination.

Keywords:

Salmonella , effector proteins, neonatal infection, enterocyte transmigration



Get-Together & Poster Viewing / Abstract-ID 250

The Importance of the RGD motif of Streptococcal protein IdeC in Streptococcus canis infection

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Streptococcus canis is an opportunistic pathogen that predominantly infects cats and dogs. Although, through contact with companion animals, human infections can also occur. *S. canis* colonizes the skin and mucosa of the host and can cause a variety of invasive diseases.

IdeC is an IgG specific protease of *S. canis*. A secreted protein acts on IgG by cleaving at the hinge region. IdeC contains an RGD motif; the most common amino acid sequence involved in adhesion to the extracellular matrix. This motif had been shown in several bacterial proteins to facilitate adhesion or invasion into host cells. The presence of this motif paired with the ability of IdeC to bind back to the bacterial surface suggests a possible role for IdeC in adhesion or invasion.

Here, recombinant protein is used to coat fluorescent latex beads, the interactions between these beads and host cells were then studied.

Based on fluorescence microscopy analysis, there is evidence that IdeC interacts with host cells in a RGD dependant manor. Further, electron microscopy indicates that IdeC coated beads are internalised by host cells.

In conclusion, IdeC may have a secondary function in bacterial adhesion and invasion into host cells.

Keywords:

Streptococcus, Adherence and invasion, RGD



Profiling the glucocorticoid receptor activation during SARS-CoV-2 infection

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Background: SARS-CoV-2 infections can result in imbalanced immune responses facilitating inflammations in severe COVID-19 cases. Glucocorticoids (GCs), such as dexamethasone, have become a standard therapy for controlling the inflammatory response. The immunosuppressive features of GCs resulted in controversies about whether GC treatment should be implemented during virus infections. Glucocorticoid receptor activation (GRA) induces an immunosuppressive state but also shows induction of autophagy and enhancement of metabolism.

Goal: As SARS-CoV-2 limits autophagy and modulates metabolism, we hypothesize direct modulations of SARS-CoV-2 to GR signaling.

Results: We show that SARS-CoV-2 infection causes an accumulation of key metabolites whereas GC-treated Calu-3 human lung cells show the opposite effect. Some GR target proteins are upregulated upon SARS-CoV-2 infection. Despite endogenous GRA, we detect COVID-19 prototypic pro-inflammatory cytokine secretion upon SARS-CoV-2 infection. GC co-treatment during infection leads to a significant reduction of cytokine secretion with comparable or even enhanced viral replication in Calu-3 cells.

Conclusion: SARS-CoV-2 infection is modulating GR signaling and immunometabolism likely to promote its own propagation. Future investigations aim at characterizing the exact virus-GR signaling host protein interactions to identify specific host targets for the development of combined antiviral/anti-inflammatory therapies.

Keywords:

SARS-CoV-2, Glucocorticoid Receptor, Immunity, Inflammasome, Autophagy



Get-Together & Poster Viewing / Abstract-ID 263

Isolation attempts of neurotropic rustrela virus

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Until recently, rubella virus (RuV; *Rubivirus rubellae*), exclusively infecting humans, was the only known virus of the genus *Rubivirus* (family: *Matonaviridae*). In 2020, two relatives of RuV were discovered in Africa and Europe: ruhugu virus (RuhV; *Rubivirus ruteetense*), the closest relative of rubella virus, found in oral swabs of presumably healthy bats, and rustrela virus (RusV; *Rubivirus strelense*) detected in brain tissue from diverse neurologically diseased mammals at several zoos in northern Germany. Recently, it was also published that the so-called staggering disease in cats is most likely caused by RusV. However, the zoonotic potential of those viruses remains unclear.

Unfortunately, several attempts to isolate RusV, including electroporation of various cells with RusV RNA, inoculation of diverse cell lines with homogenized RusV-positive brain material or co-culturing cells derived from experimentally infected RusV-positive animals with a number of neuronal or non-neuronal cells were unsuccessful.

Next attempts for rescue and isolation of RusV will include the generation of a set of recombinant rubi-like viruses by establishing a pipeline for reverse genetics and subsequent infection experiments with brain organoids and rodent-derived brain slices. This will allow further characterization of viral tropism and molecular pathogenesis and provide important insights into this newly discovered neurotropic RNA virus and its potential for zoonotic transmission.

Keywords:

neurotropic RNA virus, reverse genetics, virus isolation



Get-Together & Poster Viewing / Abstract-ID 265

RNA-based immunity against flaviviruses in arthropods and mammalian cells (RIFLA VIRAM)

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The sensing of double stranded RNA (dsRNA) by dsRNA binding proteins (dsRBP) is one of the key innate immune responses in eukaryotes. In arthropods, for example, RNA interference (RNAi) is the main antiviral defense mechanism triggered by dsRBP. In this study, we aim on the determination of the interactome of dsRNA as well as the involvement during viral infection and mechanistic characterization of dsRBP in tick-borne encephalitis virus (TBEV) infected cells of the vector tick *Ixodes (I.) ricinus*. In order to observe innate immunity in ticks, TBEV infected *I. ricinus* organ cultures will serve to obtain a greater knowledge of the pro- or antiviral properties of dsRBP identified by mass spectrometry. For this, specific RNAi screens in ex vivo tick organ cultures will be established. Midgut and salivary glands were already successfully dissected from *I. ricinus* female ticks and cultivated for up to 9 weeks. Furthermore, we could show organ viability by a resazurin based assay as well as movement of the dissected midguts. We confirmed TBEV infection and replication in midguts and salivary glands. Our results will be compared to data from human cell lines to characterize essential factors in TBEV replication cycle in vector and host.

Keywords:

dsRNA binding proteins, flaviviruses, proteomics, mass spectrometry, tick organ cultures, RNAi



Get-Together & Poster Viewing / Abstract-ID 266

The Relevance of Pim and Src kinase inhibitors in the ZIKV life cycle

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While most Zika virus (ZIKV) infections are asymptomatic or mild, several cases of congenital malformations and microcephaly have been reported in newborns and fetuses of ZIKV-infected pregnant women. To date, no effective vaccine or specific drug is available, highlighting the need of identifying antiviral drug targets for the treatment of ZIKV infection. Src family kinases (SFks) and Pim kinases regulate cell cycle, differentiation and apoptosis and play a role in controlling the innate immune response. We therefore aim to study the impact of the SFK inhibitor AZD0530 and the pan-Pim kinase inhibitor AZD1208 on the ZIKV life cycle by evaluating the effects on viral replication, subcellular localization of viral proteins and infectivity of ZIKV. Both inhibitors have antiviral potential against ZIKV, with AZD1208 eliciting stronger antiviral effects. Indeed, inhibition of SFK and Pim1-3 significantly reduces the number of infectious particles and the amount of ZIKV envelope protein and non-structural protein 1. This is also associated with a reduction in intracellular and extracellular ZIKV genomes, primarily by AZD1208. In addition, the antiviral effect is accompanied by an upregulation of several interferon-stimulated genes (ISGs) such as GBP1, PKR or ISG15, indicating an involvement of the innate immune response. Thus, we are analyzing kinome profiles to further dissect the cell signaling cascades involved in ZIKV infection and their deregulation by these inhibitors.

Keywords:

Zika Virus, Pim kinases, Src Family kinases, Cell signaling cascades



Get-Together & Poster Viewing / Abstract-ID 267

The role of epidermal growth factor receptor in the viral life cycle of the hepatitis E virus.

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Although hepatitis e virus (HEV) is a common cause of acute viral hepatitis, prevention and treatment options remain scarce. Our recent work has identified cholesterol metabolism as druggable target to combat viral infection by interfering with the viral release pathway. We aimed to dissect metabolism-regulating signaling cascades, focusing on the epidermal growth factor receptor (EGFR) signaling. Kinome analyses of persistently HEV-infected A549 cells reveal activation of EGFR, associated with a decrease in EGFR levels and a delay in the response to EGF. To determine the role of EGFR in the viral life cycle, it was modulated by siRNA-based knockdown or erlotinib-mediated inhibition. We observed an increase in released infectious viral particles, which coincided with higher levels of extracellular and intracellular HEV genomes. Using a subgenomic luciferase reporter assay, we noted enhanced viral genome replication. In addition, endolysosomal structures and actin cytoskeleton are affected by erlotinib. Kinome profiles indicated a modulation of focal adhesion-related pathways, which are linked to actin cytoskeleton organization, a key viral structure for initiation, maintenance and spread of infection. This study highlights EGFR as a novel HEV host factor. As its inhibition results in favorable conditions for viral spread, it may be a counter-indication in patients receiving EGFR inhibitors. Modulation of EGFR signaling could be a strategy to interfere with HEV life cycle.

Keywords:

hepatitis e virus, epidermal growth factor receptor signaling, erlotinib, focal adhesion



Get-Together & Poster Viewing / Abstract-ID 282

Small molecule inhibitors of HSP70 chaperones influence tick-borne flavivirus infectivity and NS1 protein secretion

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Flaviviruses are arthropod-borne RNA viruses that include one of the major tick-borne viral pathogens of humans, tick-borne encephalitis virus (TBEV). The flavivirus non-structural protein 1 (NS1) is a conserved 46–55 kDa protein that exists in different glycoforms and multifunctional oligomeric complexes. Heat shock protein 70 (Hsp70) chaperones are cellular protein folding catalysts. Part of this family is the ER chaperone binding immunoglobulin protein (BiP), a key regulator of the unfolded protein response (UPR). Flaviviruses replicate along the ER membrane and are known to activate and manipulate the host UPR. In this project, we investigate the direct interaction of flavivirus glycoproteins with BiP and its impact on downstream signalling.

We could show that infection with the tick-borne flavivirus Langat virus (LGTV) leads to induced cellular BiP expression. Furthermore, we found evidence that flavivirus NS1 proteins interact with BiP and the interaction differs between viral species. We could demonstrate that the interaction is associated with the N-glycans of the NS1 proteins. Direct interaction with the substrate binding domain of BiP is required for LGTV NS1 secretion and can be targeted by Hsp70 inhibitors with little impact on viral infectivity. In contrast, inhibition of the nucleotide binding domain of Hsp70 drastically reduces LGTV infectivity. These results suggest a key role of Hsp70 chaperones in flavivirus synthesis, assembly and NS1 secretion.

Keywords:

Tick-borne encephalitis virus (TBEV), flavivirus, ER stress, unfolded protein response (UPR), heat shock protein 70 (Hsp70), binding immunoglobulin protein (BiP)



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The immunomodulatory role of flavivirus sNS1 protein on monocyte-derived dendritic cells

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Arthropod-borne flaviviruses cause important human diseases, including encephalitis and meningitis with long-term neurological sequelae. The flavivirus non-structural protein 1 (NS1) is secreted from cells in infected individuals and known to play a role in viral pathogenesis. We recently showed that sNS1 of different flaviviruses dampen the immune response of dendritic cells upon stimulation. However, the immunomodulatory role of sNS1 during flavivirus infection is less clear. Here, we examined the immune response of monocyte-derived dendritic cells (moDCs) during infection with Usutu virus (USUV) or stimulation with polyinosinic-polycytidylic acid (poly(I:C)). Pretreatment with recombinant USUV sNS1 was performed 16 hours prior to stimulation or infection. Cell lysates were used for western blot analysis of melanoma differentiation-associated protein 5 (MDA5) expression and supernatants for detection of pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor (TNF- α) by ELISA. In poly(I:C) stimulated moDCs, cytokines and MDA5 expression were downregulated after pretreatment with USUV sNS1. Interestingly, cytokine production of moDCs was low after USUV infection, but upregulated in cells that were pre-treated with sNS1 prior to USUV infection. Our results suggest discordant effects of USUV sNS1 on uninfected bystander and actively infected moDCs. Unraveling the immune signaling in moDCs will advance our understanding of immunomodulation by flavivirus sNS1.

Keywords:

Flaviviruses, neurotropic infections, immunomodulation, arboviruses, astrocytes.



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Differential impact of SEC61B on the processing and function of filovirus glycoproteins

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Current antiviral drugs mainly target viral proteins, resulting in resistance development and the need for new antivirals. In particular, broad-spectrum are urgently needed to combat zoonotic spillover events. Generating host-directed antivirals may constitute a strategy to combat both issues and endoplasmic reticulum (ER) proteins may represent suitable targets. We assessed the potential of the ER translocation channel SEC61 as a therapeutic target. For this, we created cell lines with a knockout (KO) of SEC61B, a subunit of the SEC61 channel, and examined the effects on processing and function of glycoproteins (GPs) of zoonotic viruses. While SEC61B-KO had no impact on processing of the Ebola virus (EBOV) GP, cleavage of Marburg virus (MARV) GP was abrogated. SEC61B-KO in target cells reduced entry of particles pseudotyped with EBOV-GP while SEC61B-KO in cells producing MARV-GP pseudotypes reduced production of infectious particles. To determine whether SEC61B-KO affects viral replication, we used replication-competent, chimeric vesicular stomatitis virus (VSV) expressing EBOV-GP or MARV-GP. Both chimeric viruses but not VSV showed reduced replication in SEC61B-KO cells and an inhibitor of the SEC61 channel, Apratoxin S4, was more active against VSV-EBOV-GP and VSV-MARV-GP as compared to VSV. Although the mechanism of underlying antiviral activity remains to be fully elucidated, our data suggest that targeting the SEC61 channel may represent a viable antiviral strategy.

Keywords:

Filoviruses, Glycoproteins, ER translocation, CRISPR/Cas9

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Makorin Ring Finger Protein 2 as a target of SARS-CoV-2 Nsp3 protein

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SARS-CoV-2 utilizes host proteins to facilitate its own viral growth. In this study, we found that SARS-CoV-2 infection in Huh7 and Huh7.5 cells results in protein accumulation of Makorin Ring Finger Protein 2 (MKRN2). Yeast two-hybrid screening revealed that MKRN2 interacts with several SARS-CoV-2 viral proteins including nonstructural protein 3 (Nsp3). MKRN2 is an E3 ubiquitin ligase targeting the restriction factor p53 and the NF- κ B subunit p65 for poly-ubiquitination and the subsequent proteasomal degradation. The papain-like protease domain in Nsp3 carries catalytic activity to de-ubiquitinate host proteins. Expression of SARS-CoV-2 Nsp3 in HEK293 cells elongates the half-life of MKRN2 via de-ubiquitinating of MKRN2. As a result, Nsp3 causes protein accumulation of MKRN2 and the subsequent degradation of p53 and p65. As a consequence degradation of p65 strongly impairs NF- κ B activity which is important to induce cytokine expression.

Keywords:

SARS-CoV-2, MKRN2, Nsp3, p53, and p65

Get-Together & Poster Viewing / Abstract-ID 299

Endogenous bornavirus-like genetic elements in insectivores (order Eulipotyphla)

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Endogenous bornavirus-like genetic elements (EBL) are molecular fossils of bornaviruses in host genomes and thought to be a consequence of long-term virus-host interactions. Recent studies suggested antiviral properties of EBL in squirrels, but studies on known natural reservoir hosts of bornaviruses are sparse. The objective of this study was to identify EBL in insectivores (order Eulipotyphla). This large order of insectivorous mammals includes the reservoir host of Bornavirus 1 (BoDV-1): the bicolored-white toothed shrew, *Crocidura leucodon*. Currently available insectivore genomes were screened in silico, and revealed putative EBL in 10 of 13 species with available datasets. Intriguingly, three putative EBL derived from BoDV-1 nucleoprotein were identified in *Crocidura indochinensis*, tentatively termed ciEBLN1-3. DNA from European shrew species *C. leucodon* (Cl), *C. russula* (Cr), and *C. suaveolens* (Cs) was analyzed for respective ciEBLN homologs via PCR, Primer-walking and Sanger sequencing, and via RT-PCR and transcriptome analysis if RNA was available. Sequences detected were subsequently termed ciEBLN1-3, crEBLN1-2 and csEBLN1-2, and RNA expression of ciEBLN2 confirmed in tissue samples and shrew-derived cell cultures. Detection of EBL in *C. leucodon* warrants further in-depth studies of these elements and their function.

Keywords:

Bornaviridae, endogenous viral elements, reservoir



SARS-CoV-2 induces vital NET formation independent of available oxygen

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During COVID-19, neutrophils are activated and massively release neutrophil extracellular traps (NETs), contributing to disease severity. Yet, the exact mechanism of NET formation is not fully understood. Since patients suffer from acute hypoxia during severe cases, it is necessary to study neutrophil biology at low oxygen level. Thus, we compared NET formation of human neutrophils as response to recombinant SARS-CoV-2 viral proteins under hypoxia and normoxia (1 - 21 % O₂). Calprotectin was quantified in cell culture supernatant and found to be significantly increased by stimulation with spike protein under both oxygen conditions, while cell death marker LDH remained unaltered. Vesicular structures with positive NET marker signals were observed during confocal microscopy, indicating a vital form of NET formation, rather than cell death-linked NETosis. Electron microscopy confirmed that NET- packed vesicles were formed in the neutrophils by stimulation with spike protein. Additionally, in vivo samples were collected from SARS-CoV-2 infected hamsters at 6 days post infection. Here, lung tissue shows clear signs for hypoxia by positive staining for hypoxia inducible factor. In good correlation to the in vitro data, bronchoalveolar lavage fluid of SARS-CoV-2 infected hamsters showed similar NET- vesicles as found in response to spike protein in vitro. In conclusion, this is the first report of a vesicular NET-release as response to SARS-CoV-2-infections under hypoxic conditions.

Keywords:

NET formation, SARS-CoV-2, Hypoxia



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Plasmid conjugation in *E. coli* field isolates exposed to biocides

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Low concentrations of antimicrobials are known to increase the conjugation frequency (CF) of *E. coli*, facilitating the spread of antimicrobial resistance, while the impact of biocides on plasmid conjugation is still unclear. CFs were analyzed for suitable donor-recipient combinations of *E. coli* from livestock, food and human sources using liquid mating and subsequent enumeration of recipients and transconjugants on selective agar plates. Subinhibitory concentrations of benzalkonium chloride, chlorhexidine digluconate, octenidine dihydrochloride and glutaraldehyde were added to the mating mix and CFs were determined with and without biocide supplementation. In the mating experiments, we included two ampicillin resistant donor strains (carrying IncFII or IncN plasmids) and four recipients (carrying IncFI or pO111 or no plasmids). Chromosomally encoded tetracycline (n=3) or ciprofloxacin (n=1) resistance served as selective markers for the latter. Overall, CF mean ranged between 6.5×10^{-6} and 1.2×10^{-3} . Our findings emphasize the presence of considerable strain-specific variation and show that the exposure to biocides can influence the CF in *E. coli*. These results call for further investigation to better understand the impact of biocide exposure on the spread of antimicrobial resistance among bacterial populations.

Keywords:

antimicrobial resistance, biocide, *E. coli*, plasmid



Get-Together & Poster Viewing / Abstract-ID 216

Quantification of extended spectrum beta-lactamases producing *Escherichia coli* in broilers manure

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Chicken manure is commonly utilized as fertilizer in agriculture due to its rich nutrient content, which benefits plant growth. However, if the manure originates from a flock carrying bacteria resistant to antimicrobials, it can contribute to the spread of antimicrobial resistance (AMR) in agricultural produce, potentially affecting human health. To quantify the extended spectrum beta-lactamases (ESBL) producing *Escherichia* (*E.*) *coli* in chicken manure, we developed a Quantitative Microbial Risk Assessment (QMRA) model.

The primary objective of this research is to estimate the concentration of colony-forming units (CFUs) per gram of manure present in the barn. To achieve this, the model incorporates data such as bacterial growth rates, excretion rates, transmission between broilers and bacterial environmental decay. The QMRA simulates the whole production period, starting from day-old chicks to slaughter day. To account for uncertainties and introduce variability in the results, a stochastic approach was adopted. The outcomes of this research may have significant implications for public health, as ESBL-producing *E. coli* is the most prevalent resistant bacteria in poultry production.

Keywords:

Antimicrobial resistance, poultry production, Risk Assessment



Occurrence of multidrug-resistant high-risk clonal *Escherichia coli* lineages in the Baltic Sea with simultaneous low antimicrobial selection pressures

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Antimicrobial resistant (AMR) *E. coli* (EC) increasingly occurs in human medical, veterinary, and environmental settings. The World Health Organization categorized extended-spectrum β -lactamase (ESBL)-producing Enterobacterales among the top-priority pathogens for prospective antibiotic (AB) development. It has been shown that not only AB residues but also heavy metals/metalloids (HM) facilitate the spread of AMR bacteria across different locations, which poses a serious health risk for humans and animals in the One Health context. Here, we investigated correlations on the occurrence of ESBL-EC and AB/HM residues in the Baltic Sea in Western Pomerania. We examined 30 ESBL-EC from water samples collected over a period of one year. By combining whole-genome sequencing (WGS) with functional tests and UPLC-MS residue analysis, we characterized the isolates in detail and quantified selection pressures in water samples. Phenotypic data revealed resistances to some important ABs beyond ESBL resistance, such as ciprofloxacin and gentamicin while WGS suggested the presence of the high-risk clonal EC lineages of sequence types (ST)131, ST117, and ST58. We also detected genotypic AB and HM resistance genes, such as to aminoglycosides, arsenic, and mercury. While residue analysis is still ongoing, first results indicate overall low residue levels. Our findings highlight the importance of the Baltic Sea area as a hotspot for AMR bacteria even in the absence of high selection pressures.

Keywords:

Antibiotic Resistance; Antibiotic residue analysis; Baltic Sea region; One Health

Get-Together & Poster Viewing / Abstract-ID 258

Prevalence of Extended-Spectrum β -Lactamase Producing Enterobacteriaceae Among Clinical Isolates From Dogs Admitted to a Veterinary Hospital in Vienna

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Extended-Spectrum β -Lactamase (ESBL)-producing carbapenem-resistant Enterobacteriaceae are classified by the World Health Organisation (WHO) as pathogens of critical priority for development of new antibiotics because of their clinical importance. Companion animals may act as reservoirs of ESBL; however, data on prevalence is still scarce. To assess the situation in Vienna, 89 fecal samples from dogs visiting the small animal hospital at the University of Veterinary Medicine, including 3 samples from healthy dogs living at the University, were collected. The samples were streaked on selective media containing 2 μ g/ml cefotaxime. Species identification was carried out using MALDI-ToF. The isolates were tested for phenotypic resistance using a combination disc test by EUCAST standards. An antibiotic disc containing meropenem was added to screen for additional resistance to carbapenems. Among the 89 samples, 14 isolates of Enterobacteriaceae were ESBL, of which 12 were E.coli, one Klebsiella pneumoniae and one Enterobacter cloacae, reaching a prevalence of 15.7%. None of the isolates were resistant to meropenem. This study confirmed that there is a substantial percentage of dogs carrying ESBL in Vienna, which may pose a risk to public health.

Keywords:

Antimicrobial Resistance, Companion Animals, Extended-Spectrum β -Lactamase, One Health



Get-Together & Poster Viewing / Abstract-ID 259

Unravelling the mechanisms of heteroresistance in methicillin-resistant *Staphylococcus aureus*

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Antimicrobial resistance has emerged as a major clinical and public health challenge worldwide. However, infections of methicillin-resistant *S. aureus* (MRSA) have been increasing frequently and bacterium is also developing resistance to a wide range of antibiotics such as erythromycin, oxacillin, and fluoroquinolones. In order to develop the reliable solution to global issue of antimicrobial resistance in *Staphylococcus aureus*, there is a dire need to clearly understand the mechanism of antimicrobial resistance. Albicidin is a promising antibiotic that inhibits the DNA gyrase's activity. Gram-negative bacteria develop resistance to albicidin through several mechanisms such as albicidin degradation by endopeptidase AlbD and binding of albicidin through MerR-like transcriptional regulator AlbA. However, there is a huge gap of scientific knowledge about mechanisms of Albicidin resistance in gram-positive bacteria. The aim of the current study is to understand the mechanism of albicidin resistance in MRSA. In order to achieve the objective, the minimum inhibitory concentration of antibiotic will be determined through MIC assay and bacteria will be evolved to gradually increasing concentration of albicidin. The whole genome sequencing of antibiotic evolved and non-evolved bacteria will be performed and analyzed. The results of the study will lead to a novel mechanism of albicidin resistance in a Gram-positive bacterium.

Keywords:

Antimicrobial resistance, *Staphylococcus aureus*, Albicidin, Single nucleotide polymorphism

Get-Together & Poster Viewing / Abstract-ID 296

Hidden antimicrobial resistances in *Vibrio parahaemolyticus*: Environmental bacteria as sources or vehicles for the spread of plasmid encoded clinically important antimicrobial resistances

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AMR is on a rise and challenges global One Health. The emergence of different antimicrobial-/biocide resistances in the individual One Health compartments is usually associated with an adaption of the bacteria against prevailing selection pressures. MGEs carrying transmissible resistance determinants are common and are widely spread among bacteria. Because of their localization on plasmids, bacteriophages and insertion sequences, they can also be transmitted between bacteria by different mechanisms during horizontal gene transfer. Investigation and monitoring on the emergence of transmissible resistances is important, but sometimes their phenotypic development is masked by their hosts. Recently, a *V. parahaemolyticus* isolate was notified to carry a carbapenemase-producing plasmid, which only lead to slightly increases MIC values for carbapenems in *Vibrio* spp. Nevertheless, the location of the resistances on a conjugative plasmid result to a high resistance phenotype against different carbapenems after natural transmission into a broad range of Enterobacteriaceae isolates. The properties and the genome of the plasmid associated with this hidden resistance phenotype as well as the genotypic and phenotypic features of the *V. parahaemolyticus* isolate will be presented and discussed. The data clearly showed that some bacteria can acquire and mask resistance plasmids, which were further spread to clinically relevant genera associated with severe nosocomial infection in human.

Keywords:

AMR, plasmid, genome, transfer, carbapenem

Get-Together & Poster Viewing / Abstract-ID 302

Indications of transmission of *mcr-1.26* IncX4 plasmids along the poultry food chain to humans

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The drivers of emergence and evolution of antimicrobial resistances include antimicrobial use and abuse in human, animal and environmental sectors. Their interconnection allows the spread of resistant bacteria and resistance determinants between sectors. One such example is the transmissible plasmid-mediated colistin resistance (*mcr*) first discovered in 2016. Since then, the main determinant *mcr-1* has been found in a vast variety of plasmid backbones in diverse bacterial species across all sectors. To date, 34 variants of *mcr-1* have been described with varying prevalences. Whereas common variants, such as *mcr-1.1* can be used for quantification of transmission events, rare variants allow for epidemiological tracing-back analysis to identify the origin and transmission dynamics of these genes. *mcr-1.26* is rare and was detected in 2018 in an *E. coli* isolated from a hospitalized patient in Germany. We report on the presence of *mcr-1.26* in 16 *E. coli* and one *K. pneumoniae* originating from poultry, such as feces and retail meat, already found in 2014. The *mcr-1.26* was located on transmissible IncX4 plasmids highly similar to the plasmid reported for the human samples. Our study provides indications for the emergence in and transmission of *mcr-1.26*-carrying IncX4 plasmids along the poultry food chain. Finally, our study indicates ongoing plasmid evolution of *mcr-1.26* IncX4 by the acquisition of an additional beta-lactam resistance gene and a transposase.

Keywords:

mcr, IncX4, *Escherichia coli*, *Klebsiella pneumoniae*, colistin, horizontal gene transfer



Clostridioides difficile in Honduras, Central America: an updated genomic and phenotypic characterization.

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Clostridioides difficile is an anaerobic enteropathogen of noted clinical importance in hospital and community settings. Its ubiquitous presence in pets, animals, food products and the environment -together with its ability to form spores- favor its survival and dissemination. Some strains with possible zoonotic implications have been described over the years, and its dynamic is still under research. *C. difficile* poses a threat especially for elderly and immunosuppressed populations. In Central America, hypervirulent and multidrug-resistant genotypes have been previously reported. Thirty-one isolates from patients in two major hospitals in Tegucigalpa, Honduras were characterized using whole genome sequencing (WGS) and phenotypical antimicrobial susceptibility testing (AST). Two toxigenic PCR-ribotypes RT027 (ST1) and RT002 (ST8) were detected. All RT027/ST1 isolates (n=29) were found to be resistant to moxifloxacin, tetracycline, and linezolid, whereas RT002/ST8 isolates (n=2) were susceptible. This correlates with the presence of certain genetic elements associated with antimicrobial resistance in the analyzed strains. Worryingly, core genome MLST cluster analysis shows a close relationship between the RT027/ST1 isolates, suggesting an ongoing outbreak of multidrug-resistant *C. difficile* in both hospitals with unknown sanitary and economic implications. This emphasizes the need for a One Health research approach to develop intervention measures for control and prevention.

Keywords:

Clostridioides difficile; RT027; RT002; AMR, MDR

Get-Together & Poster Viewing / Abstract-ID 320

Antimicrobial Resistance (AMR) in *E. coli* from ducks and duck meat in Germany

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*In Germany, in 2022, AMR in *E. coli* from ducks and duck meat was investigated in a monitoring program based on Commission Implementing Decision (CID) (EU) 2020/1729. Boot sock samples were randomly collected at farm and meat samples were collected at retail. *E. coli* was isolated from the samples using routine methods. Isolates were submitted to the NRL for Antimicrobial Resistance for species confirmation and antimicrobial susceptibility testing to 15 antimicrobials as prescribed in the CID and minimum inhibitory concentrations were evaluated based on EUCAST epidemiological cut off values or similar provisional values defined by the European Reference Laboratory and EFSA.*

*A total of 158 confirmed *E. coli* isolates from farms and 149 isolates from fresh duck meat were included in the analysis. Resistance in isolates from meat samples (M) tended to be higher than from farm samples (F). Highest resistance was observed to tetracycline (M 24.2 %, F 18.4 %), ciprofloxacin (M 20.1 %, F 15.2 %) and ampicillin (M/F 18.4 %). Resistance was absent to gentamicin, amikacin and meropenem and extremely rare to 3rd gen. cephalosporins, azithromycin (1 isolate M each) and tigecycline (1 isolate F). Resistance to colistin was observed in four isolates (1 F, 3 M). More F isolates were fully susceptible than M isolates (59.5 % vs. 48.3 %, $p=0.05$).*

*Resistance of *E. coli** from ducks and duck meat was lower than in isolates from broilers and turkeys. The reason remains to be elucidated.*

Keywords:

AMR, *E. coli*, ducks, meat

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 311

Establishment of an endemic West Nile virus maintenance cycle in Berlin

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West Nile virus (WNV) is a mosquito-borne arbovirus that can cause West Nile fever (WNF) or neuroinvasive disease (WNND) in humans. Human WNV cases have been reported in Berlin since 2019, but due to reliance on passive surveillance and detection, WNV infections are likely underdiagnosed.

Here, we traced acute WNV cases transmitted to the State Office for Health and Social Affairs Berlin (SOHSA) in 2021, and analysed cerebrospinal fluid (CSF) samples from patients with encephalitis of unknown aetiology for WNV. Mosquitoes were trapped at identified exposure sites and examined for WNV. We characterized two acute WNV cases without travel history in Berlin in 2021, a blood donor with WNF and a patient with WNND, and identified one WNND case retrospectively from CSF material. WNV was also identified in two *Culex pipiens* mosquitoes collected at one exposure site. In 2022, monitoring at the same site confirmed presence of WNV in five *Culex pipiens* mosquitoes. Phylogenetic analysis of WNV genomes from our study and other WNV sequences show that the sequences from Berlin form a monophyletic clade containing two unique single nucleotide variants (SNVs). The WNV sequences from one patient and a mosquito shared an additional SNV.

Overall, our study provides evidence WNV has established an endemic maintenance cycle in Berlin with autochthonous human WNV lineage 2 infections. Since cases are expected to increase, enhanced surveillance, vector management and public awareness are needed.

Keywords:

West Nile Virus, Berlin, mosquitoes

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 203

Human land-use change alters immunogenetic-pathogen links in a generalist rodent and zoonotic reservoir

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Anthropogenic land use modifies landscapes and the surrounding matrix of natural habitats, alters biodiversity and increases the contact probabilities between wildlife, domestic animals and humans. Numerous lines of evidence further suggest that anthropogenically transformed environments increase the emergence of zoonoses. Wildlife species, however, respond to human disturbance differently. Generalists have high plasticity, can occupy a broad ecological niche, and thus, can adapt to human landscape modifications. Yet, whether anthropogenic disturbance modifies host-pathogen co-evolutionary relationships in generalists is unknown. We assessed pathogen diversity, neutral genome-wide (SNPs) and adaptive MHC class II diversity in a rodent generalist inhabiting three lowland rainforest landscapes with varying anthropogenic disturbance, and determined which MHC alleles co-occurred more frequently with 13 gastrointestinal nematodes, blood trypanosomes, and four zoonotic viruses. Pathogen-specific selection pressures varied between landscapes. Genome-wide diversity declined with the degree of disturbance, while MHC supertype diversity was only reduced in the most disturbed landscape. Furthermore, pristine forest landscapes had more functionally important MHC-pathogen associations compared to disturbed forests. We show impoverished co-evolutionary links between host and pathogen owing to human disturbance even in generalists and suggest this may facilitate host switching by pathogens.

Keywords:

Habitat disturbance, generalist species, spiny rat *Proechimys semispinosus*, genome-wide and MHC class II diversity, host-pathogen interactions, co-infections, OneHealth

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 228

Role of a novel pilus protein in DNA uptake and natural transformation of *Campylobacter jejuni*

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Campylobacter jejuni is a major food-borne pathogen causing acute gastroenteritis and occasionally severe long-term complications. The diverse population structure, particularly caused by natural transformation, is considered the basis for adaptation and survival. To visualize natural transformation capacity, *C. jejuni* were incubated with fluorescently labelled DNA and treated with DNase. Imported DNA in single cells was detected and quantified. Around 35 % of cells took up DNA, visible as distinct foci in single bacteria. Since the mechanism of uptake is not fully understood, we created mutants lacking proteins with potential roles in uptake. The outer membrane pore PilQ was essential for DNA uptake and the inner membrane channel ComEC for natural transformation. The periplasmic DNA binding protein ComE was negligible for uptake. Intriguingly, a mutant lacking the unique pilin-like protein Cj0683 was nearly abolished for DNA uptake. However, the rare observed DNA uptake events contained similar amounts of DNA than those of the wild-type. We identified Cj0683 as novel pilus-like protein, essential for the efficient initialization of DNA uptake. It is tentative to speculate that Cj0683 might be part of the competence (pseudo)-pilus, grabbing external DNA, thereby initializing DNA import events over the outer membrane in *C. jejuni*. Unravelling features important in natural transformation might lead to target identification, reducing the adaptive potential of pathogens.

Keywords:

natural transformation, genetic diversity, adaptation, reduction strategies, food safety

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 232

Mathematical modeling of mosquito borne diseases in Germany

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Usutu virus (USUV) has emerged as a public health concern in Europe, showing a rapid spread and impact on avian populations. We propose a mathematical model to characterize the transmission dynamics of USUV between *Culex* mosquitoes and European blackbirds. Our model incorporates mosquito population dynamics, driven by temperature, rainfall, and wind speed. We analyzed the model using mathematical techniques to gain insights into the dynamics of USUV transmission. Through sensitivity analysis, we investigated the influence of key parameters of the mosquito offspring number and the basic reproduction number, on the spread of the virus. We extend the model to include control measures targeting the mosquito population. Numerical simulations are conducted to assess the effectiveness of these control measures. By integrating epidemiological, ecological, and environmental factors, our model offers a comprehensive understanding of USUV transmission dynamics between mosquitoes and birds in Germany. The insights derived from this study can guide surveillance strategies, inform evidence-based public health policies, and aid in implementing targeted interventions to mitigate the impact of USUV on avian populations in Germany. In conclusion, this research provides a valuable tool for decision-makers to develop proactive strategies for the prevention and control of USUV, ultimately protecting public health and preserving the well-being of avian populations in Germany.

Keywords:

Flavivirus, reproduction number, offspring number, vector control

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 208

Human-to-human transmission of Andes hantavirus modeled in the Syrian hamster

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Hantavirus induced cardiopulmonary syndrome (HCPS), a sporadic yet lethal zoonotic disease, is caused by Andes virus (ANDV, species *Andes orthohantavirus*), which is prevalent in South America. Historically, ANDV spillover was thought exclusive of human exposure to aerosolized infectious particles released from the excreta and/or secretions of a natural reservoir (i.e. wild rodents). However, increasing cases of human-to-human transmission have been reported in the last years. Unfortunately, very scarce information exists about this mode of transmission. Syrian hamsters are the only animals that can recapitulate human pathogenesis of ANDV-HCPS and have been critical to study different aspects of this pathology. However, neither shedding nor transmission has ever been investigated in this animal model. Here, we inoculated hamsters with ANDV and routinely sampled oral and rectal mucosa and opportunistically urine. During the experiment we exposed naïve animals to inoculated animals by direct contact to assess potential ANDV transmission and to compare patterns of infection, shedding and disease progression between cohorts. ANDV-RNA was detected via all routes sampled from infected animals of both cohorts, to similar titers, and the virus was transmitted efficiently to >40% of the naïve animals. Furthermore, we detected a transmission chain event and chronic shedders. Overall characteristics of infection and disease between infected animals did not differ between cohorts.

Keywords:

Hantavirus, Hantavirus pulmonary syndrome, Syrian hamster, Human-to-human transmission

Session 5: Environmental factors & Ecology of Zoonotic Infections / Abstract-ID 239

Integrative early-warning modelling of West Nile virus transmission in Germany

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Driven by globalisation and climate change, mosquito-borne viruses have emerged in Europe over the last decades, with West Nile virus (WNV) transmission detected in Germany in 2018. *Culex pipiens* is considered the primary vector, but the sister species *Cx. torrentium* also plays a role in WNV transmission, especially in Central Europe where both species occur in sympatry. Assessing WNV transmission risk often relies on mechanistic or correlative models, but approaches that integrate both streams for more informative results are lacking in the literature. Moreover, most models use static data, limiting their suitability for real-time predictions. This study developed a “hybrid” model in which estimates of mosquito abundance refine a mechanistic R0 model based on temperature-dependent transmission parameters. Mosquito abundance was derived from real-time surveillance data collected from traps across Germany, allowing a vector-to-host ratio parameter to be incorporated in the model. Hourly updated nation-wide climate data was used as input to yield short-term forecasts presented as risk maps, serving as a tool for risk assessment. The findings suggest that the role of *Cx. torrentium* in WNV transmission was underestimated, as its high vector competence for WNV generated high R0 values. Integrative models that take advantage of regularly updated climate and mosquito surveillance data could greatly enhance decision-making regarding surveillance plans and preventive measures.

Keywords:

West Nile virus, modelling, early warning systems, *Culex torrentium*, mosquito-borne diseases

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 312

Advancing European research on viral zoonoses through the new One-Health-driven programme, “Integrated Services for Infectious Disease Outbreak Research” (ISIDORE)

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ISIDORE is a new approach to epidemic preparedness and response research in Europe. It assembles and provides free access to an unprecedented One Health-driven integrated portfolio of cutting-edge research resources, dedicated to the study of any epidemic-prone disease, including viral zoonoses. ISIDORE involves all the major European Research Infrastructures and networks in the field of biomedical research, from the most fundamental (e.g. structural biology) to the most applied (e.g. vaccine development and clinical trials).

Readiness to future infectious disease is now the main scope of the ISIDORE programme. Launched in November 2022, the preparedness programme includes a list of priority preparedness pathogens, established using One Health criteria, to prioritize zoonotic diseases of greatest concern. Clustered in four calls for proposals, it is aligned with the strategies of key leading European and international Public Health, animal health, and research and innovation bodies, specifically those in the initiative’s strategic advisory board: CEPI, the European Commission, the ECDC, EFPIA, EMA, FAO, GloPID-R, HERA, WHO and WOAHA. This EU-funded mechanism enables transdisciplinary projects to be conducted, to improve preparedness, and accelerate innovation during times of emergencies. We will showcase the results of ISIDORE-supported projects and ongoing opportunities for free access open to all researchers.

Keywords:

One health, pandemic preparedness, zoonoses, research infrastructure, EU, service.

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 289

Mosquito ID: sequencing out of the mobile suitcase lab as an early warning system for emerging infectious diseases

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Mosquito-borne diseases are responsible for a yearly spillover to around 700 million people. Early warning surveillance tools are needed to prevent disease spread to humans and animals in times of climate change and globalization. The aim of this study was to develop a field deployable sequencing platform to identify potential mosquito pathogens, species and host from blood meals. A rapid extraction reverse purification method was developed using mosquito specimens collected in the field and/or from laboratory colonies in Greece (Athens) and Spain (Barcelona), including *Culiseta longiareolata*, *Culex pipiens*, *Aedes albopictus*, *-cretinus* and *-aegypti*. Nucleic acids from the specimens were isolated using a rapid “all-in-one” extraction protocol based on lysis buffer, glass beads, magnetic beads, heating and vortexing. Oxford Nanopore Technologies rapid barcoding sequencing was performed using a MinION MK1C device. A reverse transcription step was performed for RNA targets. All steps were carried out in the fully equipped suitcase lab. A specific offline BLAST database was created to semiautomatically identify mosquito species, host in blood meal and pathogens. The species was correctly identified in all samples. Both animal and human DNA could be detected in the mosquito blood meal. The protocol performed in the suitcase lab allows fast mosquito “footprint” analysis directly in the field, allowing an early warning for mosquito-borne diseases and on-site outbreak investigation.

Keywords:

mosquito borne diseases, nanopore sequencing, early warning system

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 237

Strengthening One Health research capacity in Guinea through characterization of acute febrile illness of unknown etiologies

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Strong One Health (OH) research platforms are a cornerstone of pandemic preparedness. While many pathogens underlying acute febrile illnesses (AFI) are zoonotic, animal and OH research targeting AFI is limited and the underlying infectious agents often remain underdiagnosed. In 2022, we started the implementation of an interdisciplinary study to characterize AFI in Guinea, the Fever Project. A core objective of the project is to strengthen capacities for OH research and zoonotic disease risk reduction. The Fever Project consists of two parallel strands of questionnaires and specimen collection: 1) hospital patients presenting with acute fever, and 2) healthy community members and animal species commonly found in and near communities. Fundamental premises of the project are capacity strengthening and collaboration and coordination with national and local Authorities, to ensure that the project’s research activities successfully contribute to national and regional epidemic preparedness and health security efforts. The Fever Project research platform has already provided opportunities for other OH collaborations, leveraging our collaborations and partnerships. We trained 25 Guinean partners during three workshops and three field-based trainings on laboratory and field techniques. The project also explicitly includes annual stakeholder meetings, for dissemination of information, solicitation of input, and to conduct programmatic evaluation of our collaborative research approach.

Keywords:

capacity building, research platforms, acute febrile illness, Africa

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 298

Seroprevalence of anti TBE-IgG and anti-NS1-IgG in blood donors in a highly endemic TBE area in south-eastern Germany

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Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Central Europe. Since the introduction of an effective vaccine no seroprevalence studies have been possible. Therefore, no actual data on the incidence and prevalence of TBE infection in a population is currently available.

We developed an ELISA to detect IgG antibodies against NS1 antigen of TBEV indicating recent or past infection. Using this new test, we tested 1.300 sera from blood donors against TBEV IgG antibodies differentiating between vaccine-induced and infection-induced IgG antibodies.

1.300 sera were screened by a conventional TBE-IgG ELISA. Positive sera were re-tested by NS1-IgG ELISA and by TBEV neutralization test (NT) to distinguish between vaccine-induced and infection-induced antibodies. The NT was applied to exclude IgG cross reactions with other flaviviruses, e.g. yellow fever vaccination, West Nile infection or dengue infection.

The preliminary results show a TBE-IgG prevalence of 85%. Of these, 2,6% reacted positive against TBEV-NS1-IgG, indicating past infection. Most of the other TBEV-IgG positive sera reacted positive in the TBEV NT indicating past vaccination against TBE and excluding other flavivirus infections or vaccinations.

Our data indicate a much higher vaccination rate as reported by official public health data. About 2% of all blood donors and about 10% of non-vaccinated blood donors exhibit serological evidence of a past TBEV infection.

Keywords:

tick-borne encephalitis, incidence, vaccination, highly endemic region

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 317

Assessment of treatments to reduce the amount of antibiotic-resistant bacteria in chicken manure

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Spreading of antibiotic resistance is one important threat for human health. Microorganisms with resistance deteriorate the effectiveness of antibiotics, which are the first choice in controlling and treating infectious diseases. There are three main ways to transfer antimicrobial resistance. Due to high selection pressure, antibiotic resistance more often develop and spread in hospitals, is transmitted between animals and spread into the environment. The project ENVIRE focuses on the transfer of resistant bacteria from chicken farms to humans through the environment.

Chicken manure is a valuable nutrient source for plants and therefore often used as agricultural fertilizer. However, it can contain high amounts of antimicrobial resistant bacteria. Those stay there during the fertilizer production process. Therefore, it is the aim of the study to find manure treatment conditions under which most of those bacteria will be eliminated.

In this study, we consider the two processes with highest relevance in fertilizer production: anaerobic and aerobic fermentation. Anaerobic fermentation is a sustainable process that is used for biogas production like an alternative energy source. In aerobic fermentation many parameters can be varied to find the most effective in fertilizer production and microbiological reduction.

This study could be very useful for the chicken production industry because the results of the research will help to reduce the spread of antibiotic resistant bacteria.

Keywords:

Antibiotic resistant, Antimicrobial resistant bacteria, Chicken manure, Anaerobic digestion, Anaerobic fermentation (Composting)

Session 6: Public Health & Pandemic Preparedness / Abstract-ID 181

Knowledge, attitudes and behavior towards tick prevention and tick-borne diseases – a survey among Lyme borreliosis cases in Bavaria in 2019

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Since no vaccination against Lyme borreliosis (LB) is available, individual protective measures are prevention options. However, application depends on knowledge and perceptions. We asked LB cases about their knowledge, attitudes and behavior (KAB) regarding tick prevention and tick-borne diseases to inform future prevention campaigns.

We invited a subset of LB cases reported between weeks 23–35 in 2019 in Bavaria to participate in a KAB survey. We send invitations ≤ 2 weeks after notification.

Of 1,105 cases invited, 377 participated (34%); 298 were adolescents/adults, 79 were children (median age 59 and 6 years, respectively). 67% (221/328) noticed the tick bite that likely led to the current LB. During summer, 84% spend time outdoors at least multiple days a week. Participants were misinformed, thinking ticks fall from trees (60/377, 16%), vaccination protects against LB (48/377, 13%) and no tick-specific repellents exist (48/377, 13%) or they were not sure they exist (129/377, 35%). Though most believe checking for ticks after time outdoors, wearing long clothes, wearing closed shoes and tugging pants in socks are effective in protecting against tick bites, fewer apply those measures regularly (99% vs. 72%; 93% vs. 40%, 88% vs. 51%; 85% vs. 17%).

Although most participants were well-informed, existing misinformation had effects on perceived effectiveness and the application of protection measures. Information campaigns should specifically address identified misinformation.

Keywords:

lyme disease, KAB survey, ticks, health literacy, prevention

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity / Abstract-ID 261

Investigating the biomarker potential of host proteins and development of lateral flow assays to detect *Mycobacterium bovis* infection

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Mycobacterium bovis (*M. bovis*), a globally prevalent pathogen, causes zoonotic tuberculosis (zTB) in humans and bovine tuberculosis (bTB) in cattle; with significant public, animal welfare and economic impact. While efficient control measures in cattle in some countries rely on test and cull, the field under-performance of diagnostics is a significant challenge. We screened a panel of host immune proteins; and developed up-converting reporter particle (UCP) based lateral flow assays (LFAs); which have proven applications in human TB diagnostics.

Samples from naïve and *M. bovis* experimentally challenged cattle with or without prior BCG vaccination were tested by ELISA. Levels of bovine tuberculin (PPDb) specific IL-2, CXCL10 and CCL4, in addition to IFN- γ , showed promising biomarker potential to not only identify *M. bovis* infection but also enabled Differentiation of *M. bovis* Infected animals from BCG Vaccinated Animals (DIVA).

UCP-LFAs were developed to detect six bovine proteins (IFN γ , IL-2, IL-6, CCL4, CXCL9 and CXCL10). PPDb specific levels of IFN γ , IL-2, IL-6, CCL4 and CXCL9 determined by UCP-LFAs discriminated *M. bovis* challenged animals from naïve (area under the curve [AUC] range: 0.87-1.00) and BCG vaccinated animals (AUC range 0.97-1.00). This is the first report of UCP-LFA technology for bTB detection. This builds to our on-going efforts of developing a robust, user-friendly multi-biomarker test (MBT) with enhanced diagnostic accuracy for bTB and zTB diagnosis.

Keywords:

Biomarkers, bovine tuberculosis, chemokines, cytokines, diagnostics, DIVA, upconverting reporter particles, UCP-LFA

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity / Abstract-ID 226

Emerging crop pathogenic fungi *Fusarium* threatens hatching success in freshwater turtles – The impact of healthy egg microbiomes in pathogen resistance

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The *Fusarium solani* species complex (FSSC), a large genus of globally distributed filamentous fungi found predominantly in soil, decomposed organic matter and plants, are well known for causing invasive diseases in humans (fusariosis) and severe economic losses of crops (e.g. cereal, cacao, oil palms). They occur as trans-kingdom pathogens and are listed as fungal priority pathogens by the WHO since 2022. For about a decade, FSSC has been known to cause fusariosis in sea turtle eggs, leading to hatching failure and mass hatchling mortalities worldwide. In 2019, we reported for the first time fusariosis infections in a freshwater turtle species, the yellow-spotted Amazon River turtle (*Podocnemis unifilis*), which inhabits a pristine environment in the Ecuadorian Amazon (Carranco et al. 2022, Transboundary and Emerging Diseases). Differences in the microbial composition of symptomatic and asymptomatic eggs suggest that *Fusarium* pathogens interact with the internal egg microbiota. Moreover, the egg microbiome is influenced by river sand and water environment (Carranco et al. 2022, Mol Ecol), which shapes the microbial composition and impacts egg health. With this prior knowledge we show the critical role of the host-associated internal egg microbiota in hatching success, pathogen resistance, and turtle health. Taken together, host-associated microbiota remains a neglected component of the One Health framework, albeit their significant role for host and environment health.

Keywords:

fungal infections, fusarium pathogens, fusariosis, egg microbiome, host-pathogen interactions, disease resistance

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity / Abstract-ID 309

MERS-CoV–Specific T-Cell Responses in Camels after Single MVA-MERS-S Vaccination

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Middle East respiratory syndrome coronavirus (MERS-CoV), closely related to SARS-CoV and SARS-CoV-2, emerged on the Arabian Peninsula in 2012 provoking severe respiratory diseases and multi-organ failure in humans. It reproductively circulates in dromedary camels as natural reservoir with recurrent spill over into human population. As classical One Health concept, broad vaccination of the camels is a promising approach to reduce viral shedding within the animal population and consequently also to humans. MERS-CoV specific T cells are described to be crucial for rapid viral clearance and for vaccine-induced balanced immunity. In a first proof-of-concept-study, dromedary camels were vaccinated once with MVA-MERS-S under field conditions and in presence of natural MERS-CoV infection. The recombinant MVA is a safely tested and well established orthopoxvirus delivering the full-length MERS-CoV spike protein. Seropositive camels resulted to mount substantial increase in MERS-CoV specific T cell immunity after single vaccination demonstrating profound immunogenicity of our MVA-based vaccine candidate.

Keywords:

MERS-CoV, dromedary camels, vaccination, MVA

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity / Abstract-ID 243

Biological attack causing plague? *Yersinia pestis* used in simulation exercise for the investigation of an alleged use of biological weapons.

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Yersinia pestis, as the causative agent of plague, poses a potential threat to national security and public health and is therefore classified by US Centers for Disease Control and Prevention to the highest alarming level, a so-called Category A organism. The pathogen has been the subject of research in various bioweapons programs since World War II. A deliberate aerosol release of *Y. pestis* would result in a rapidly increasing number of cases with respiratory symptoms and, without effective treatment, a high mortality rate. However, the pathogen is endemic in some areas of the world. Independent investigations with watertight evidence are needed to distinguish between biological attacks and natural outbreaks. The United Nation's Secretary-General's Mechanism (UNSGM) is the only independent mechanism that can be initiated to investigate the alleged use of biological weapons. Simulating these types of threats is essential to be responsive in the event of an actual intentional release. Recently, biological weapons experts, nominated by UN member states, were given the opportunity to investigate a simulated plague outbreak in a comprehensive field exercise. In addition to mission planning, collection of evidence in the field and reporting the findings, interaction with stakeholders such as the UN and analytical laboratories also played an important role. The so-called Capstone Exercise is one of the German efforts to strengthen the operational readiness of the UNSGM.

Keywords:

UNSGM, *Yersinia pestis*, Biological weapons, Simulation exercise

Session 7: One Health Intervention Methods + Risk Assessment & Biosecurity / Abstract-ID 183

The bat-borne influenza A virus H9N2 exhibits a set of unexpected pre-pandemic features

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Over the past years, bats have gained increasing attention as hosts for several emerging viruses of zoonotic concern, including Marburg, Ebola, SARS and MERS coronaviruses. Paradoxically, however, bats have long been neglected as a potential reservoir for influenza A viruses (IAVs). In 2017, a novel H9N2 IAV was isolated from fruit bats in Egypt. In the following years, similar viral sequences were also detected in bats in South Africa, suggesting a widespread circulation of bat H9N2 in Africa.

Here, we combined various *in vivo* and *in vitro* approaches to evaluate whether bat H9N2 is of zoonotic concern. Intranasal infection of ferrets demonstrated that bat H9N2 replicates efficiently in the upper respiratory tract and is rapidly transmitted to naive contact animals. Moreover, we found that bat H9N2 is able to replicate in human lung cultures to similar titers as human-adapted IAVs and that the human population lacks humoral immunity to bat H9N2. We also tested the ability of bat H9N2 to overcome restriction by human MxA, a crucial innate antiviral factor for zoonotic IAVs. While bat H9N2 was potently suppressed in MxA-overexpressing cells, infection of MxA-transgenic mice resulted in viral lung titers comparable to those of wild-type B6 mice. Western blot analysis revealed suppression of MxA induction in the MxA transgenic mice. Collectively, our data show that bat H9N2 meets key criteria for pre-pandemic IAVs.

Keywords:

Bat influenza A viruses, IAV, H9N2, pandemic potential, risk assessment, MxA escape, ferret transmission, lack of humoral immunity

Session 8: Antimicrobial Use and Resistance / Abstract-ID 270

Bridging the research gaps on antimicrobial resistance in sub-Saharan Africa - A One Health approach

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Disease outbreaks and management are a huge challenge for public health systems worldwide. The excessive use of drugs in veterinary and human medicine leads to a reduction in effectiveness and even to the development of antimicrobial resistances (AMR). Monitoring AMR as well as the coinfection with neglected tropical diseases (NTDs) remains a significant challenge especially across sub-Saharan Africa. The aim of this project is to strengthen the capacity across 7 Sub-Saharan countries for improved management of AMR and NTDs. The focus lies on identifying the linkages and transmission of AMR in a One Health context. In order to better control AMR, academic and research institutions from the eight participating countries have investigated and developed 6 work packages (WPs) to build the local capacity to identify the main transmission routes.

The WPs include screening for AMR in humans and livestock; investigating relationships between helminthic infections and drug resistant bacteria; developing capacities for point of need diagnostics using mobile tests for field use; identifying any changes in antimicrobial use and AMR incidence during the COVID-19 pandemic; controlling communicable disease transmission and building capacity for sustainable leadership in antimicrobial stewardship (AMS).

With the established, diverse consortium this project proposes unique solutions for AMR/AMS through the development of both knowledge and technological infrastructure.

Keywords:

antimicrobial resistance, surveillance, neglected tropical diseases

Session 8: Antimicrobial Use and Resistance / Abstract-ID 225

From heteroresistance to resistance: a single nucleotide polymorphism (SNP) homogenizes population plasticity of gene amplification based heteroresistance

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Introduction

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

Material & Methods

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

Results

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

Conclusion

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

Keywords:

heteroresistance, plasticity, ceftazidime, *Enterobacter* spp.

Session 8: Antimicrobial Use and Resistance / Abstract-ID 229

Pim-kinase inhibitors as antivirals against SARS-CoV-2

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During the SARS-CoV-2 pandemic, several treatment strategies arose relying on viral target structures and therefore pose risks of emerging escape-mutations. We therefore screened kinase inhibitors as a strategy to modulate the host. These were selected on the basis of previously observed interference with the FGF7-singaling. These inhibitors are used in cancer therapy targeting EGFR, FGFR, SFKs or Pim-kinases and were screened for their antiviral potential against SARS-CoV-2. These were selected. All inhibitors reduced viral RNA, viral proteins and virion release in a dose-dependent manner. Especially the Pim1/2/3-inhibitor AZD1208 led to a complete removal of viral traces, thus being most promising. Importantly, it was also capable of significantly reducing a fully established infection. Gene-expression analyses indicated that AZD1208 drives inflammatory and ROS-related processes. Additionally, a detailed kinome-analysis suggested the PI3K/Akt-pathway and related routes as driver of this effect. This was further validated with analyses of AZD1208-mediated effects in the presence or absence of different inhibitors of this and other pathways.

We identified Pim-kinases as tremendously potent drug-targets for a host-modulatory antiviral against SARS-CoV-2. This seemingly is based on changes in endolysosomal signaling and host-defense mechanisms. Further studies will decipher the underlying processes in more detail. Finally, inhibitors will be tested against SARS-CoV-2 in vivo.

Keywords:

Pim-kinases, antivirals, SARS-CoV-2, PI3K-Akt-signaling

Session 8: Antimicrobial Use and Resistance / Abstract-ID 278

Investigating conjugative AMR plasmid maintenance using CRISPR-Cas-based plasmid curing

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Bacterial resistance against antibiotics has become commonplace in medical, veterinary, and environmental settings. This development is particularly driven by conjugative plasmids carrying antibiotics resistance genes, which spread easily in environments of high bacterial density. Understanding the dynamics of spread of AMR genes among commercially raised animals is a crucial component of preventing spread to humans. We therefore sought to investigate the contribution of non-AMR genes on the maintenance of conjugative plasmids by curing environmental *E. coli* isolates of their conjugative AMR plasmids and comparing the resulting plasmid-cured variants to the parent strains. Here, we develop and refine a CRISPR-Cas9-based approach to curing conjugative AMR plasmids carrying ESBL or pAmpC genes using a recombinant plasmid, pCBL (Curing Beta-Lactamases). This modular system can address common challenges of curing conjugative plasmids, such as toxin-antitoxin systems and broad resistances. We employ pCBL to produce plasmid-cured variants of ESBL/pAmpC-carrying *E. coli* strains isolated from broiler chickens and their environment and characterise these strains. Current results showing differences in growth and biofilm formation in some strains will be presented. We aim to provide a fast and reliable method of curing AMR plasmids to assess the physiological impact of plasmid loss in a broad range of isolates and to investigate non-resistance advantages of conjugative AMR plasmids.

Keywords:

Plasmid Curing, ESBL, *E. coli*

Session 8: Antimicrobial Use and Resistance / Abstract-ID 297

Joint analysis of antimicrobial resistance data from human and veterinary sector - results from the project Connect One Health Data

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In the Connect One Health Data project, routine data on resistance testing in Lower Saxony from the zoonoses monitoring (ZooMo; veterinary sector) are analysed together with data from the antibiotic resistance monitoring in Lower Saxony (ARMIN; human sector) for the years 2018-2021. Proportions of resistant isolates per antimicrobial agent in the different populations are determined.

The following germ were included in the analyses: E. coli, Enterococcus faecalis, Enterococcus faecium, and MRSA. For each germ, antimicrobials for which test results were available from both sectors were included for joined analyses.

For MRSA as an example, 15 antimicrobials could be included in the analysis. In ZooMo, 217 MRSA isolates were tested for all antimicrobials, compared to 98 - 14,460 tested isolates in the ARMIN data, where the number of tests varied by antimicrobial agent. About 20% of human MRSA isolates (n = 13,077) are resistant to tetracycline, in contrast to about 90% of tetracycline resistant broiler isolates (n=15).

When interpreting the data, the background of sampling must be taken into account: fixed sampling design of healthy animals along the food chain (ZooMo) vs. passive surveillance of human patients (ARMIN). In addition, laboratory methods and interpretation standards differ between the sectors. Therefore, we are currently analysing the impact of the use of the different standards on the proportions of resistance.

Keywords:

surveillance, interpretation standards, MRSA, E. coli, Enterococcus

Session 8: Antimicrobial Use and Resistance / Abstract-ID 179

Pathology as read-out for efficacy testing of the benzothiazinone BTZ-043 against *Mycobacterium tuberculosis* in a guinea pig (*Cavia porcellus*) model

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Background and objectives: Tuberculosis (TB) is the leading bacterial cause of human death worldwide. The proportion of antibiotic resistant *Mycobacterium tuberculosis* (Mtb) strains is increasing. The aim of this study was to analyze the effect of an orally administered anti-TB drug candidate - benzothiazinone (BTZ) - 043 on Mtb induced granulomas.

Material and Methods: Eighteen guinea pigs were infected s.c. with 1×10^3 Mtb H37Rv. Starting 14 d after infection, subgroups (n=6, each) were orally treated with BTZ-043, isoniazid, and vehicle, respectively, daily for 28 d. On day 42 after infection, animals were euthanized and a complete autopsy with macroscopic assessment of lesions was conducted. Fixed (4 % formaldehyde) tissue samples and granulomas were processed by histochemical and immunohistochemical methods. Stained tissue sections were scanned as whole-slide images to allow quantification and statistical testing of lesion parameters.

Results: The extent of subcutaneous granulomas and the proportion of necrosis were significantly reduced in BTZ-043-treated guinea pigs compared to vehicle controls. Systemic spread with granuloma genesis in other organs did not occur in this group. A highly significant reduction in mycobacterial load in subcutaneous granulomas, draining lymph nodes and spleen was demonstrated.

Conclusions: BTZ-043 is a promising antibiotic and showed significant efficacy in the guinea pig model of TB after only 28 days of oral administration.

Keywords:

BTZ-043, guinea pig, *Mycobacterium tuberculosis*, MDR-TB, treatment, new antibiotics

Lunch & Poster Viewing / Abstract-ID 212

Comparison of neutralising activity of vaccine-induced TBEV antibodies against 10 genetically different TBEV strains of the European subtypes

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Vaccination against TBEV has been shown to be highly effective. However, differences in the neutralization activity of antibodies have been observed in vaccinated individuals. The aim of this study was to investigate the neutralizing capacity of vaccine-induced antibodies against 10 different TBE-virus strains after anti-TBEV IgG standardisation. 36 sera from participants were obtained and divided into three groups: 1 Encepur, 2 FSME-IMMUN, 3 Mix (participants who had received at least three vaccine doses, at least one per brand). All sera exhibited high avidities. The 12 sera from each group were titrated by ELISA and adjusted to 250 VU/ml. Then the adjusted sera were tested by micro-neutralization assays against the 10 TBEV strains using a fix antibody concentration and varying virus concentrations (10¹ to 10⁵ virus particles/ml). No significant differences were found between the groups. Variations within the groups, related to individual sera, were not associated with factors such as age or vaccination history of the individuals. Neutralizing activity was dependent on the viral strain used or on individual factors of the vaccinated individuals. The neutralizing activity did not differ based on the vaccines used. However, a vaccination history during which both vaccines were used seems to lead to a more uniform antibody response against the different viral strains than in participants who had received only one brand of vaccine.

This project was funded by Pfizer Pharma GmbH.

Keywords:

TBEV, FSME-IMMUN, ENCEPUR, AVIDITY

Lunch & Poster Viewing / Abstract-ID 240

Control of *Coxiella burnetii* in a dairy goat herd by vaccination of the offspring

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Coxiella burnetii is a bacterium that causes Q fever. Ruminants are considered to be the main reservoir and they can excrete the pathogen via birth material and milk. Humans become infected by inhaling contaminated dust and aerosols. In a dairy goat herd with an acute Q fever outbreak, all goats were vaccinated with a *C. burnetii* vaccine (Coxevac®). In the following years, exclusively female offspring were vaccinated before their first breeding due to the occurrence of side effects (skin swellings) in multiparous goats after repeated vaccine applications. Infection was monitored over four years by collecting vaginal swabs, serum samples, monthly bulk tank milk samples, and dust samples from the milking parlour. *C. burnetii* DNA was detected in vaginal swabs in each age group, mostly at lower levels (Cq>30). After vaccination, older goats showed a strong IgG phase I response, while yearlings generally reacted less intensely. Dust samples from the milking parlour (Cq 20-39) and bulk tank milk (Cq 23-43) tested positive for *C. burnetii* DNA. Vaccination boosted the natural immune response of older goats in the long term. Although vaccinating offspring alone can help to control infection in positive dairy goat herds, it does not completely prevent *C. burnetii* shedding. The extent to which the low-level detection of *C. burnetii* DNA in the samples maintains infection within the herd needs further investigation. Funded by the BMBF under project numbers 01K11726B/01KI2008B.

Keywords:

bulk tank milk, dust samples, IgG, phase-specific serology, Q fever, zoonosis



Lunch & Poster Viewing / Abstract-ID 256

Immunogenicity and efficacy of a MVA vaccine expressing spike and nucleocapsid antigens against SARS-CoV-2 challenge infection in the k18-hACE2 mouse model

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An important countermeasure against infectious diseases is the development of protective and safe vaccines. This has been demonstrated by the COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this context, the combination of different target proteins within a candidate vaccine seems to be an interesting approach to further improve vaccination strategies. The aim of this study was to characterize the immunogenicity and efficacy of Modified Vaccinia virus Ankara (MVA) based candidate vaccines expressing either the SARS-CoV-2 S-protein (MVA-S) or the SARS-CoV-2 S- and N-protein (MVA-S/N) in the k18-hACE2 mouse model.

Groups of mice were vaccinated with MVA-S or MVA-S/N comparing a prime and prime-boost vaccination regime, followed by SARS-CoV-2 infection. All animals were monitored for clinical symptoms and the viral loads in lung and brain tissue. In addition, we also characterized the SARS-CoV-2 specific immune responses.

MVA-S and MVA-S/N robustly protected the mice against SARS-CoV-2 challenge after prime-boost and also after single vaccination. We confirmed the robust activation of neutralizing antibodies and the activation of S-specific T cells after vaccination. Interestingly, MVA-S/N vaccination appears to induce slightly improved protection in these mice. Ongoing work focuses on the identification of potential immune correlates of this improved protection.

Keywords:

SARS-CoV-2, MVA, Antibodies, T cells



Lunch & Poster Viewing / Abstract-ID 301

Evolutionary inferences of *P. vivax* Duffy Binding Protein II (Pvdbp- II): The Indian setting

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The requirement of an efficient *P. vivax* vaccine is crucial as evidenced from reports of drug resistance globally. PvDBP-II, a leading vaccine candidate for *P. vivax*, has cleared Phase I clinical trial and is reported to be highly polymorphic, which might be a major obstacle on the way of attaining a successful vaccine. India, a significant contributor to *P. vivax* malaria burden in the WHO-SEAR, exhibits varying *P. vivax* endemicity. Hence, understanding the pattern of diversity and selection in Pvdbp in India would be vital for developing a DBP-based vaccine.

Genetic diversity and natural selection of PvDBP-II was investigated in 73 *P. vivax* isolates collected from different parts of India. Out of a total of 57 SNPs identified, 18 were non-synonymous and 3 were synonymous mutations. The overall nucleotide diversity of 73 PvDBP-II isolates was 0.00609 with 22 haplotypes ($Hd=0.87$) identified. The high ratio of non-synonymous to synonymous mutations suggests that PvDBP-II had evolved under positive selection.

Polymorphisms of PvDBP-II shows that isolates from India were genetically diverse. Also, findings from this study further confirmed that mutations and natural selection might increase and sustain evasion of host immunity. These results expand our understanding of *P. vivax* evolution in India and, more crucially, solidify the rationale for the development of a blood-stage *P. vivax* malaria vaccine.

Keywords:

Malaria, India, *P. vivax*, PvDBP-II, genetic diversity, natural selection

Lunch & Poster Viewing / Abstract-ID 322

Antigenic cartography using variant-specific hamster sera reveals substantial antigenic variation among Omicron sub-variants

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SARS-CoV-2 has developed substantial antigenic variability. As the majority of the population now has pre-existing immunity due to infection or vaccination, the use of experimentally immunized animal sera can be valuable for measuring antigenic differences between virus variants. Here we immunized Syrian golden hamsters by two subsequent infections with one of eight SARS-CoV-2 variants. Sera were titrated against 14 SARS-CoV-2 variants and the resulting antigenic distances visualized using antigenic cartography. The antigenic map shows a condensed cluster containing all pre-Omicron variants (D614G, Alpha, Delta, Beta, Mu, and an engineered B.1+E484K variant), and a more distinct positioning of a selected panel of Omicron sub-variants (BA.1, BA.2, BA.4/5; the BA.5 descendants BF.7 and BQ.1.18; the BA.2.75 descendant BN.1.3.1; and the BA.2-derived recombinant XBB.2). Some Omicron sub-variants were as antigenically distinct from each other as the wildtype is from Omicron BA.1. The results highlight the potential of using mono-specifically infected hamster sera for the continued antigenic characterisation of SARS-CoV-2.

Keywords:

SARS-CoV-2, Antigenic Variation, Omicron

Lunch & Poster Viewing / Abstract-ID 327

In vitro characterization of two MVA candidate vaccines expressing the fusion or matrix proteins of Nipah virus

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Nipah virus (NiV) is an emerging zoonotic pathogen from the Paramyxoviridae family that is transmitted to humans from Pteropus bats directly or via intermediate hosts. The host species has a wide distribution in Asia and the virus can infect a wide range of animals. NiV infection in humans causes severe disease characterized by acute respiratory syndrome and encephalitis and has a high mortality rate. As no treatments are available, safe and effective vaccines are needed for prophylaxis in humans and animals. We aimed to generate and characterize recombinant Modified Vaccinia virus Ankara (MVA) candidate vaccines expressing NiV fusion protein (MVA-NiV-F) or NiV matrix protein (MVA-NiV-M). The modified target genes were cloned into MVA vector plasmids and introduced into the MVA genome by homologous recombination. Recombinant MVA viruses were generated by serial plaque passaging and were amplified to high titer stocks to obtain vaccine preparations. In vitro characterization of the two candidate vaccines was performed in compliance with standardized quality control procedures. We could confirm genetic stability, unimpaired protein expression and replicative capacity in chicken embryo fibroblasts (CEF) of our two candidate vaccines. In the future, we plan to test the immunogenicity of the MVA-NiV-F and MVA-NiV-M candidate vaccines in a preclinical mouse model.

Keywords:

vaccines, vaccinia virus, Nipah virus

Lunch & Poster Viewing / Abstract-ID 207

The evolution of ecological niches between 2000-2020 of *Aedes albopictus* and six *Anopheles* species within the extended Mediterranean area due to land use and climate change

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As climate and land use have a significant impact on the distribution of mosquitoes, the Mediterranean region may face major problems with vector-borne diseases in the future. The Mediterranean is considered a hotspot of climate change due to rising temperatures, a greater precipitation variability and, in some regions, reduced rainfall amounts. Especially the temperature increase within the cold season favors the establishment of invasive species such as *Aedes albopictus*. In contrast, extreme precipitation events or reduced annual rainfall amounts prevent the spread of invasive species or favor the retreat of native species. Changes in land use, such as urbanization, deforestation or irrigation, can alter the vector distributions as well. As the Mediterranean is the “kitchen garden of Europe”, irrigation is a common practice to compensate for the lack of rainfall during the dry season.

Using Boosted Regression Trees (*Anopheles*) and MaxEnt (*Aedes*), we analyzed the change of ecologically suitable areas within the extended Mediterranean area for *Aedes albopictus* and six native *Anopheles* species between 2000 and 2020. Both models consider time series of mean and extreme climatic variables derived from the ERA5-Land reanalysis dataset and land use categories from the LUCAS land cover dataset. Finally, we want to determine whether recent changes are related to climate or land use changes and which variables are the key factors for the changes.

Keywords:

Mosquito-borne diseases

Climate change

Land use change

extended Mediterranean are



Lunch & Poster Viewing / Abstract-ID 217

Listeria monocytogenes in biofilms under conditions simulating the meat processing environment

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Listeria monocytogenes (Lm) is a foodborne pathogen of major concern for public health and the food industry. It can persist in processing environments, leading to recurring cross-contamination of food products. The biofilm lifestyle is considered to protect Lm, facilitating their survival even under harsh conditions. Various environmental stress factors influencing the biofilm forming ability (BFA) of Lm have been investigated, but the adaptation of biofilm models to conditions prevailing in food processing facilities is often neglected.

Our aim is a step-by-step approximation to conditions of the meat processing environment. Starting with a static model, 33 field isolates, sampled in official controls in Germany and mainly associated with meat production, were tested for their capability to form biofilms at 21°C. The selected isolates covered a high diversity including various MLST CCs, serogroups and genetic lineages, as well as infection- and food-related, proven persistent and atypical (low motility) Lm. We observed isolate-specific differences in the formed biofilm biomass. The majority of isolates were weak biofilm formers (20). Only 9 Lm showed a moderate BFA, but without similarities regarding the mentioned properties. Non-motile isolates displayed no BFA. Lowering the model temperature to 12°C (meat processing temperature) decreased the total biofilm biomass and delayed biofilm formation. Temperature pre-conditioning also had a negative effect on the BFA.

Keywords:

Listeria monocytogenes, biofilm, persistence

Lunch & Poster Viewing / Abstract-ID 247

Surface and wastewater as reservoirs for a broad spectrum of health related klebsiellae? Insights into the diversity and potential impact of isolates from natural and human-associated sources in Haiti

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Klebsiellae are nosocomial pathogens increasingly notified by public health services. Due to their high adaptability and their ability to acquire foreign DNAs, human infections with MDR-isolates are challenging to treat. In addition, environmental klebsiellae are also reliable indicators for the dynamics in resistance acquisition in ecosystems caused by pollutions forcing the adaptation of these bacteria. As comprehensive information from different ecosystems is lacking, klebsiellae from surface/wastewater were investigated in Haiti. Here, we report on 58 *Klebsiella*-isolates collected from 12 stations during an environmental survey of *Vibrio* in 2021, ten years after the beginning of the local cholera outbreak. Isolates genome profiling showed a broad variety of XbaI-PFGE pattern. Phenotypically, only some isolates exhibit resistances to the tested antimicrobials, which were shown to be in good agreement with the resistance genes determined by WGS. In addition, some of the isolates further exhibit a strong hypermucoviscosity. In silico dissection of the klebsiellae genomes provides a detailed insight into the genetics and their potential impact for human health. Dissection of environmental *Klebsiellae* provides information about sources of antimicrobial resistance acquisition, hotspots for the evolution of the bacteria and the general occurrence of clinically relevant lineages, which may affect the health of the local human population due to the colonization of susceptible people.

Keywords:

Klebsiella, AMR, genome, diversity, detection, environment



Lunch & Poster Viewing / Abstract-ID 253

A mechanistic model to predict spatial-temporal patterns of *Culex pipiens* s.s./*Cx. torrentium* in Germany

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Mosquitoes are well known for their ability to transmit pathogens. These include a variety of arthropod-borne viruses (arboviruses) of medical and veterinary interest. Due to globalization and climate warming, the threat of (re)emerging arboviruses is increasing in Europe. This also applies to temperate regions, where the transmission of viruses is becoming possible due to an increase in ambient temperature, i.e. shortening the extrinsic incubation period. *Culex pipiens* s.s. and *Culex torrentium*, commonly found in and around human settlements, are the primary vectors of Usutu virus and West Nile virus in Germany. The prediction of the spatial-temporal occurrence of these mosquito species is needed for the assessment of arbovirus transmission risk and to timely organise intervention methods, such as vector control. On the basis of a pre-existing model, a mechanistic model was developed to predict the spatial-temporal occurrence of *Cx. pipiens* s.s./*Cx. torrentium* in Germany. The model output is driven by local rainfall and temperature data downloaded from the Open Data Server of the German Meteorological Service. In a nation-wide field study in 2021, population data on *Cx. pipiens* s.s./*Cx. torrentium* was collected and used to evaluate the model prediction. This evaluated mechanistic model can be used to simulate vector control measurements or the impact of increasing temperatures in cause of climate warming.

Keywords:

Vector
Ecology
Mechanistic Model



Lunch & Poster Viewing / Abstract-ID 275

Chances and limitations of environmental sample matrices in avian influenza virus surveillance

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The octo-segmented, single-stranded RNA influenza A viruses can be differentiated into 18 HA and 11 NA subtypes. The majority of them can be found in water birds which constitute the natural reservoir of avian influenza viruses (AIV). Based on the type of host protease processing of the viral hemagglutinin glycoprotein, phenotypes of high (HP) and low (LP) pathogenicity can be distinguished among AIV of subtypes H5 and H7. HPAIVs induce excessive mortality in avian hosts and may comprise zoonotic propensity as well. Improved HPAIV surveillance is implicit in early warning strategies to prevent incursions into poultry production and exposure of human hosts. Here, results from field investigations for AIV in different environmental matrices are reported.

Surface water samples of 10 L water were ultrafiltrated but enrichment of AIV from spiked samples was limited to a median factor of 15 only. Although AIV genome was detected by RT-qPCR in 60% of surface water but with low viral loads. Similar results were found for sediment samples from the same water bodies. Even less successful were attempts to detect AIV RNA in environmentally deposited avian feces (median 4% positives). In comparison, about 80% of the avian carcasses retrieved in the same region tested positive for HPAIV in the same period.

The use of environmental samples for AIV surveillance cannot be recommended although the matrices, especially sediments, bear potential to inform about deposited influenza virus RNA.

Keywords:

Influenza, Water, environment

Lunch & Poster Viewing / Abstract-ID 284

Detection of “VBNC-Campylobacter” in Broiler Farm Environments: Insights from PMA-qPCR Analysis

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Studies suggest that Campylobacter can enter a viable but non-culturable (VBNC) state. The significance of this state form for a spread on farms is unclear. To investigate this, a PMA dye-supported viability qPCR approach was used to examine environmental matrices on culturable Campylobacter and “VBNC-Campylobacter” in broiler farm environment and in an experimental animal pen.

Seven visits to broiler farms were conducted, collecting environmental and barn samples. Sampling was also done in an animal facility after removing Campylobacter-positive animals. Chicken manure from five trials was examined for 72 hours. Campylobacter was cultured and if this failed, putative VBNC cells were analyzed using live-dead discrimination.

At three sampling times no Campylobacter at all was found inside or outside the chicken barn in all 72 samples. At the other four sampling times, it was present in the barn and also in environmental samples from outside (15.9% positive for “VBNC-Campylobacter”, 62.2% Campylobacter DNA-positive, 1.2% culturable *C.jejuni*).

In the experimental animal facility manure from five chicken groups was negative for culturable *C.jejuni* after 24 h. In contrast, after 72 h “VBNC-Campylobacter” were found in many manure samples.

“VBNC-Campylobacter” was found in broiler barns’ environment and inside a barn during experiments. However, further research is needed to determine long-term sustainability in broiler farms.

Keywords:

Campylobacter, viable-but-non-culturable

Lunch & Poster Viewing / Abstract-ID 304

Host preference of mosquitoes (Diptera: Culicidae) collected in Germany

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Next to vector competence, blood host preference is a major factor determining the risk pathogen transmission by mosquitoes between different groups of vertebrates.

We analysed blood-fed mosquito females, collected throughout Germany from 2016 to 2022, for their bloodmeal origin. Two PCR protocols were used: if DNA amplification and sequencing of a conserved 16S rDNA region did not yield results, the samples were subjected to COI barcoding. The obtained sequences were aligned with GenBank, and only results with a minimum identity of 97% were accepted. Of 485 mosquito specimens processed, 355 belonging to 27 species/species groups produced interpretable results. Forty-two vertebrate species were identified as hosts, including 22 mammalian, 17 avian, 2 reptilian and 1 amphibian species. Except for *Culex territans*, which exclusively fed on reptiles and amphibians, all mosquito species had fed on mammals. Five mosquito taxa could be linked to both avian and human blood hosts and 16 taxa to both non-human mammalian and human hosts.

The study demonstrates a surprisingly broad host acceptance. Both *Cx. pipiens* biotype *pipiens* and *Culiseta morsitans/fumipennis*, which are commonly considered ornithophilic, were shown to have fed on mammals, including humans. The findings suggest that host preference is generally less pronounced and bridge vectors between animals, in particular birds, and humans, may occur more frequently and widely distributed than previously discussed.

Keywords:

mosquitoes, vector, blood meal, host preference



Lunch & Poster Viewing / Abstract-ID 325

Patterns of mosquito species biodiversity in Europe based on current and future environmental suitability

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Non-native and native vector mosquito species in Europe are concerned with increasing health risks of mosquito-borne diseases in regions regarded as MBD-free under climate change. The role of mosquito diversity is unclear. For effective control strategies to prevent MBD, updating and predicting the distribution of MBD vectors in Europe under current and potential future climatic conditions is crucial. This study aims to evaluate the current and future distribution of the area suitable for mosquito species (vectors and non-vectors) in Europe, estimate biodiversity hotspots, and assess environmental niche overlaps of mosquito species. We use the ecological niche modeling approaches (MaxEnt) to estimate projections of suitable habitats for over 60 mosquito species in Europe. We used occurrence records from literature and open databases (GBIF, Vectorbase, Mosquito alert) and relevant environmental variables. We highlighted the important environmental covariates influencing mosquito species distribution. We compare current and future distributions, specifically in the 2050s and 2070s, using the latest emissions scenarios based on “Shared Socioeconomic Pathways” (SSPs) from the Intergovernmental Panel on Climate Change. Our findings contribute to understanding mosquito species distribution in Europe, a crucial step towards effective decision-making for mosquito control and disease prevention strategies.

Keywords:

vector mosquito species, ecological niche modeling, MaxEnt, biodiversity hotspot, niche overlap, climate change, public health, Europe



Lunch & Poster Viewing / Abstract-ID 326

Impact of host genetics in the house mouse hybrid zone on microbiome composition and antimicrobial resistance

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Antibiotic resistance (AR) is a priority public health problem. Selection mechanisms for AR are well understood, while the transmission of antibiotic resistance genes (ARGs) is a comparatively under-researched topic. Particularly, the links between molecular, genomic, bacterial community, and host community level are rarely analysed in an overarching manner. Given the importance of understanding how bacteria carrying ARGs interact in the microbiome and with the environment of their hosts, here we used an amplicon sequencing approach, to simultaneously study bacteria and predict ARGs composition in natural populations from house mice (*Mus musculus*). We compared gastrointestinal bacterial diversity, composition and abundance across a gradient of pure and hybrid genotypes in the European house mouse hybrid zone between the subspecies *M. m. musculus* and *M. m. domesticus* at different geographical and temporal scales. We detected extreme phenotypes of bacterial abundance with the hybridisation of mice. Some bacteria and overall ARGs have elevated abundance in hybrid genotypes. In contrast, the abundance of other bacteria, but not of any ARGs, are reduced in hybrids compared to parental mice. Our results confirm that host genotype drives the abundance of gastrointestinal bacteria and raises the question whether environmental covariates of the hybrid zone or host genotypes influence the occurrence of ARGs in natural populations of house mice.

Keywords:

ARG, Mice, Genetics, Microbiome

Lunch & Poster Viewing / Abstract-ID 182

Tularemia in Germany — A rare disease?

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Francisella tularensis is a facultative intracellular bacterium causing the zoonosis tularemia in humans. Germany is a low incidence country with regard to tularemia in humans, about 80 cases are annually reported since 2019, but showing an increase of notifications over the last 20 years. However, tularemia is assumed to be underdiagnosed and underreported in Germany. Among the notified cases, the most frequent clinical presentations of tularemia were the glandular and ulcero-glandular form (45 %), the pneumonic form (12 %) and the oropharyngeal form (5 %). In addition, we will describe some particularly interesting typical (hunt-associated outbreak) or seldom (squirrel bite) cases of human tularemia. The pulmonary form of tularemia could be mistaken for lung cancer, and one of these cases will be presented here.

In Germany, two basal clades of *F.tularensis* ssp.holarctica (Fth) are predominantly responsible for human and animal tularemia: isolates belonging to basal clade B.6 (biovar I, erythromycin-sensitive) or isolates of basal clade B.12 (biovar II, erythromycin-resistant). We will report on the genotypic diversity of both clades in Germany. Generally, Fth clades are not well characterised and clade-specific phenotypes are poorly understood. However, we will demonstrate that the two basal clades exhibit distinct protein expression profiles and a clade-specific growth behaviour in liquid media.

Keywords:

Tularemia, *Francisella tularensis*, phylogenetic analysis

Lunch & Poster Viewing / Abstract-ID 221

The influence of single amino acid exchanges on the sensitivity of rapid antigen tests illustrated by the example of the N protein of SARS-CoV-2

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Introduction: Even after the pandemic has subsided, monitoring of SARS-CoV-2 variants is necessary to respond rapidly to the emergence of possible new highly contagious forms of the virus. Rapid antigen tests (RATs), which proved their worth during the pandemic, are well suited for this purpose. However, this requires that they are also able to recognize variants with substitutions of individual amino acids.

Objectives: The main target for SARS-CoV-2 RATs is the nucleocapsid protein (N), which has fewer mutations compared with the spike protein (S), but amino acid exchanges also occur in this protein, which may lead to a change in the binding epitopes of the detection antibodies in the RATs. Therefore, we investigated whether amino acid exchanges in the SARS-CoV-2 nucleocapsid protein can affect the sensitivity of RATs from different suppliers.

Methods: We constructed multiple recombinant protein mutants (mirroring specific amino acid exchanges SARS-CoV-2 nucleocapsid proteins), by prokaryotic expression and site-directed PCR mutagenesis. Sensitivity of RATs was tested by loading different amounts of coronavirus proteins into the RATs according to a generalized protocol.

Results: We were able to confirm the loss of sensitivity of some RATs to detect N proteins with single amino acid exchanges. Therefore, we recommend the use of RATs with SARS-CoV-2 variant adapted detection antibodies.

Keywords:

SARS-CoV-2

Rapid Antigen Tests

Amino acid exchange

Sensitivity of Assays



Lunch & Poster Viewing / Abstract-ID 252

Everything under control – Effects of combining an insect-specific virus and an insecticide in mosquito larvae to control mosquito populations

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The risk of disease outbreaks caused by mosquito-borne arboviral infections is becoming increasingly problematic, even in so far temperate regions. This is due to climate change, globalization, and urbanization, which favour the spread of vectors and the pathogens they transmit. Vaccination or medical treatment is often not possible for arboviral diseases, making vector control essential for effective disease control. The unrestricted use of chlorinated insecticides (notably DDT) in the era of malaria control not only led to the development of resistance in vectors, but also had dramatic effects on human and animal health and severely damaged the environment. A modern approach is a more species-specific vector control by using entomopathogenic microorganisms and their active compounds. In our study, we targeted larvae of *Culex pipiens molestus* with the insect-specific virus Culex Y virus (CYV) in combination with an insecticide. CYV replicates in mosquitoes and specifically in their larvae, but not in ecologically important insects. We tested CYV-injected mosquito larvae and the progeny of infected mosquitoes with the insecticide spinosad at three different concentrations. Preliminary results suggest that offspring of CYV-infected mosquitoes exposed to spinosad had lower survival rates than the control group. The results could serve as a basis for developing integrated pest management strategies that combine biological control with reduced use of synthetic insecticides.

Keywords:

Mosquito larvae, Culex Y virus, Vector control, Spinosad, Culex pipiens molestus

Lunch & Poster Viewing / Abstract-ID 272

Anti-SARS-CoV-2 Seroprevalence among Seasonal Field Workers in Lower Saxony – Risk-Perception and Protective Behaviour

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Seasonal field workers (FW) are an at-risk group for SARS-CoV-2 (SC2) infection. Outbreaks of SC2 among FW were reported in Germany (D). The study aimed to estimate prevalence of SC2-IgG (SC2-P) among FW and to assess perception of risk for SC2 to develop targeted public health measures.

In 2021 and 2022 sera from FW at two farms in Lower Saxony (LS) were tested for SC2 by ELISA. Data on demographics, risk behaviour, disease awareness and housing conditions was collected by a multi-lingual questionnaire.

189 FW (median age 45 years, 58% male) from Poland (PL) (59%), Romania (RO) (18.0%) and D (23%) participated. SC2-P of 34 (18%) FW corresponded to status after infection; SC2-P of 34 (18%) FW indicated status after vaccination. 19 (56%) FW reported previous infection with SC2; 21 (62%) FW reported vaccination. Out of 140 FW 49% lived in single rooms; 6% shared rooms with more than 3 people. 46% FW reported having been in quarantine (40%) or isolation (6%). FW adhered to preventive measures e.g. frequent handwashing (98%), ventilation (95%), masks (93%) and social distancing during work (90%) or leisure (85%). On a scale of 1 to 6, FW from RO scored higher for personal risk for SC2 (4.9) than FW from D (3.4) or PL (2.6).

SC2-P among FW in LS was 18%; FW from RO had lowest SC2-P. Data indicate that 44% of infections had not been diagnosed. Due to workplace arrangements FW had a high adherence to preventive measures. Data will be used to develop public health measures for FW.

Keywords:

SARS-CoV-2, seroprevalence, field workers, prevention, risk, Public Health



Lunch & Poster Viewing / Abstract-ID 285

Rapid assembly of MERS-CoV genomes using a yeast-based reverse genetics system

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The Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic in Africa and the Middle East. While MERS-CoV phylogenetic clade C strains circulate among African dromedary camels, clade B strains continuously cause human spillover in the Arabian Peninsula. To enable functional comparison, we used the transformation-associated recombination (TAR)-cloning methodology to clone the full genome of distinct MERS-CoV lineages into yeast-artificial chromosomes. Briefly, 11 primer pairs were designed to bind conserved genomic regions of all MERS-CoV lineages (clades A, B and C), allowing amplification of viral RNA either from camel or human samples. PCR products were assembled into a pCC1-His vector using highly transformable *S. cerevisiae* VL6-48N. After successful yeast transformation, correctly cloned MERS-CoV genomes were amplified in *E. coli* 10G electrocompetent bacteria and sequences were verified through Oxford Nanopore Sequencing. Plasmids containing MERS-CoV genomes of interest were purified, linearized, *in-vitro* transcribed, and transfected into BHK-J cells. Recombinant MERS-CoV isolates were rescued and purified for subsequent functional assays. Overall, this method allows to quickly generate recombinant MERS-CoVs within 2-3 weeks. Moreover, it facilitates complex site-directed mutagenesis in individual genomic regions, enabling rapid characterization of new MERS-CoV variants emerging in camels and humans.

Keywords:

MERS-CoV
TAR

Lunch & Poster Viewing / Abstract-ID 294

Host cell entry efficiency and neutralization sensitivity of SARS- CoV-2 XBB sublineages

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The SARS-CoV-2 XBB variant has dominated the COVID-19 pandemic, with the vast majority of cases occurring in either the XBB.1.5 or XBB.1.16 sublineages globally. We used neutralizing antibodies used for COVID-19 therapy or patient plasma, cell lines, and rhabdoviral pseudovirus particles (pp) carrying SARS-CoV-2 spike (S) proteins to test the ability of XBB.1.5 and XBB.1.16 to enter cells and evade antibodies. In comparison to XBB.1pp, XBB.1.5pp and XBB.1.16 pp showed enhanced cell entry and were less susceptible to inhibition by anti-ACE2 antibody. Except for Sotrovimab, XBB.1.5pp and XBB.1.16pp were highly resistant to neutralization by monoclonal antibodies. Finally, the plasma from quadruple-vaccinated individuals who received monovalent or bivalent vaccine boosters as fourth vaccination, as well as triple-vaccinated individuals with breakthrough infection during the BA.5 wave in Germany, was highly and comparably resistant against neutralization by antibodies. According to our research, SARS-CoV-2 lineages XBB.1.5pp and XBB.1.16pp have similar host cell entry efficiency, cell line tropism, and neutralization evasion. We are currently using reverse genetics systems to create isogenic viruses including XBB.1.5 and XBB.1.16 and will investigate their replication in nasal epithelial cells to better understand why XBB.1.5 and XBB.1.16 are outcompeting other descendants of the XBB.1 lineage.

Keywords:

SARS-CoV-2, XBB, Neutralization



Lunch & Poster Viewing / Abstract-ID 321

Epidemiology of SARS-CoV-2 infections in Ghana: A Cross-Sectional Study from April to June 2022 in Kumasi

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Introduction: Data on COVID-19 is still lacking in many parts of Africa. To bridge these gaps, we studied the epidemiology of SARS-CoV-2 in Kumasi, Ghana from April to June 2022.

Methods: We sampled individuals visiting the Kwadaso SDA hospital COVID-19 Testing Center, Kumasi and collected data on socio-demography, clinical symptoms and vaccination status from 83 people. Nasopharyngeal swabs and sera were obtained. Viral RNA was extracted and tested with a pan-Sarbecovirus real-time RT-PCR. Samples with Ct ≤ 30 were sequenced. Serum samples were tested for antibodies against spike (S) and nucleocapsid (N) proteins by ELISA.

Results: Participants comprised patients (47%), hospital staff (20.5%) and international travelers (32.5%) who were aged 31-40 years and mostly males (67.5%). Vaccination rate was 72.5% and RNA positivity was 42.2%. We could not demonstrate that vaccination prevented infection (OR = 1.38, CI: 0.48 – 4.01, p = 0.553). Four BA.4 (22A) and one BA.5 (22B) Omicron variant sequences were obtained. Antibody positivity was 65% for S only and 35% for both S and N.

Conclusion: The high vaccination rate observed, compared to 57.3% for Ghana may be due to the composition of healthcare workers and international travelers. BA4 and BA5 were co-circulating relatively early compared to many countries in Europe and the lack of vaccine effectiveness for symptomatic infection reflects their immune escape properties. Protection against severe infection could not be assessed.

Keywords:

Omicron, Vaccination, RT-PCR, Kumasi, SARS-CoV-2

Lunch & Poster Viewing / Abstract-ID 332

Synergistically targeting pyrimidine metabolism and RNA integrity for the treatment of respiratory diseases caused by zoonotic influenza A viruses

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The zoonotic potential of this virus has been highlighted by recent transmission events of avian influenza viruses (AIV) circulating in wild and domestic birds to different mammalian species, and sporadic transmissions of swine influenza viruses (SIV) to humans. High mutation rates and reassortment of the segmented influenza viruses require yearly updates of human vaccines. In addition, rising levels of viral resistance affect therapeutic efforts for humans suffering from severe respiratory symptoms. To counteract this pathogen, we are exploiting antiviral drug combinations that not only rely on viral targets, but also on host factors. We have therefore combined a nucleoside analogue, N4-hydroxycytidine (NHC; active compound of Molnupiravir), with a pyrimidine synthesis inhibitor targeting the enzyme dihydroorotate dehydrogenase (DHODH), to achieve exquisite synergy against virus propagation. AIVs of H5 subtypes as well as different SIVs have been tested in vitro for their sensitivity against different drug combinations. So far, we found all tested strains to be sensitive to drug treatments at varying degrees. To further analyze this drug efficiency in vivo, animal trials in the ferret and swine model are planned. In conclusion, our approach may lead to the development of drugs that are effective against influenza A viruses with a low susceptibility to the development of viral resistance, and are therefore encouraging in terms of preparedness for future influenza pandemics.



Lunch & Poster Viewing / Abstract-ID 186

Black-headed gulls as a reservoir for multidrug-resistant high-risk clonal Enterobacterales

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Wildlife and the environment are important hotspots for the accumulation of multidrug-resistant Enterobacterales (MDR-E). They not only harbor opportunistic but clinically relevant pathogens that are additionally often resistant to heavy metals (HM).

Here, we explore MDR-E in black-headed gulls from nature conservation areas in Western Pomerania and characterize their resistance and virulence geno- and phenotypes over three years. Overall, we collected 799 fecal samples in 2021 and 2022. These included adult birds, nestlings, and collective fecal samples. We screened the samples for extended-spectrum beta-lactamase-producers on chromogenic agar plates with cefotaxime. All putative positive strains were whole genome-sequenced and analyzed for the carriage of additional antibiotic and HM resistances as well as virulence genes. Sampling for 2023 and phenotypic screenings are currently ongoing.

Overall, we detected twelve (2021) and 29 (2022) MDR-E. Most strikingly, we found the international high-risk clonal lineage sequence types 38, 131 and 744 to be present in both sampling seasons, which were, however, non-clonal. A majority was MDR and carried HM resistance genes and pronounced virulence features.

In conclusion, black-headed gulls breeding in Western Pomerania are an important reservoir for MDR, clinically relevant Enterobacterales. Our study highlights the importance of the One Health approach considering the interdependence of human, animal, and environmental health.

Keywords:

AMR bacteria, ESBL, One Health, wild birds



Lunch & Poster Viewing / Abstract-ID 189

Single Point Mutations in the Outer Capsid Protein VP4 Improve Rescue of Simian Rotavirus Reassortants Carrying Antigens of a Human Rotavirus

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Rotavirus A (RVA) is a non-enveloped virus with a segmented RNA genome, which can cause severe diarrhea in a broad range of hosts including wild animals, livestock and humans. In order to generate next generation vaccines against a broad range of RVA types, a reverse genetics system based on a simian rotavirus strain was utilized here to exchange the antigenic capsid proteins VP4, VP7 and VP6 with that of human rotavirus field strains. One triple-reassortant was successfully rescued, but replicated only poorly in the first cell culture passages. However, viral titer enhanced steeply upon further passaging. Next generation sequencing of the passaged virus revealed a single point mutation (A797G) resulting in an amino acid exchange (E263G) in VP4. After introducing this mutation into the original VP4-encoding plasmid and repeating the rescue experiment, the triple-reassortant replicated to high titers already in the first cell culture passage. However, introduction of the same mutation into the VP4 of other human RVA strains did not improve the rescue of those reassortants, indicating strain specificity. Further long-term passaging experiments are ongoing, which identified one further mutation (A95G) in the VP4 segment, possibly involved in enhanced replication. The results indicate that specific point mutations in the VP4 can improve the rescue of recombinant RVA reassortants in cell culture, which might be useful for targeted generation of novel vaccine strains.

Keywords:

Rotavirus A; reverse genetics system; reassortment; point mutation; capsid proteins



Lunch & Poster Viewing / Abstract-ID 268

Expansion of raccoons (*Procyon lotor*) threatens already endangered native animal species and poses health risks for humans

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Background and aims

Raccoons (*Procyon lotor*) are considered as an invasive alien species (IAS) in Europe. Due to its omnivorous diet, adaptiveness and the lack of natural enemies, it has been spreading widely and will continue in the future.

The aim of this study was to investigate the impact of raccoons on protected or endangered native species, as well as on the economy and animal and human health, the role as a vector of parasites and pathogens and its increased spread in urban areas.

Methods

234 raccoons were dissected to investigate diet composition and parasite fauna. 229 raccoons blood and swab samples were taken to test for viral infections in collaboration with the Friedrich-Loeffler-Institute (FLI). Current distribution and land use was used to model possible future distribution.

Results

A predation on endangered and protected native species, (e.g. yellow-bellied toad (*Bombina variegata*)) and birds could be proven through stomach content analyses. The parasite fauna shows a high number of parasite species from different classes. The key species is *Baylisascaris procyonis*, that appeared in 95% of the samples. Some of the blood samples were tested positive for WNV (West Nile Virus) and USUV (Usutu-Virus) which reveals a vector-competence of raccoons for this pathogens.

Conclusions

Raccoons can have strong negative impacts on native species like amphibians and birds as well as on animal and human health caused by the spread of zoonoses.

Keywords:

Raccoon (*Procyon lotor*), invasive species, metazoan parasite fauna, *Baylisascaris procyonis*, *Plagiorchis muris*, zoonotic diseases, impact biodiversity



Lunch & Poster Viewing / Abstract-ID 271

Raccoon Dogs as Zoonotic Vectors

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Background

Raccoon dogs *Nyctereutes procyonoides* are native to Asia but increasingly occur in Europe. The introduction in Europe was caused by anthropogenic influence which classifies it as an invasive alien species. These IAS are known for having an impact on native ecosystems based on their role as vectors of parasites and pathogens as well as predation of native species. The aim of this study was to reveal carried parasites and pathogens of raccoon dogs from Germany. The results are used to assess the raccoon dog's impact on native ecosystems.

Methods

73 raccoon dogs were examined by dissection and feces examination. The stomach content was separated. Found species were identified morphologically as well as genetically. Based on the results, the prevalence, intensity and abundance of parasite infestation was calculated.

Results

Based on the diet, a predation on native animal species such as the protected frog *Rana temporaria* could be shown. In total, 9 ecto- and 11 endoparasite species could be identified. Highest prevalence was found for *Uncinaria stenocephala*, highest intensity was found for *Echinococcus multilocularis*.

Conclusions

The present study shows that *Nyctereutes procyonoides* could play an important role in the spread of zoonoses, because it serves as host for a high number of parasite species. He can also cause a decline in native animal species and therefore has a negative impact on native ecosystems and on animal and human health.

Keywords:

raccoon dog
invasive alien species (IAS)
zoonoses
vector
parasites
wildlife

Lunch & Poster Viewing / Abstract-ID 291

Pool party? West Nile virus neuroinvasive disease in a captive common seal (*Phoca vitulina*)

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In Germany, the first West Nile virus (WNV) infections were discovered in birds and horses in 2018 and since continue to be registered with distinct predominance in Eastern Germany. Additionally, 48 human infections were recorded between 2019/2022. In 2020 evidence was found that WNV is established in local mosquito populations (*Culex pipiens* complex).

While a number of wildlife bird species carry and succumb to WNV, so far - beside horses - no other mammal species was diagnosed with WNV infection in Germany.

In August 2022 a male adult common seal (*Phoca vitulina*) from a zoo had loss of appetite. Seven days after onset the animal showed mild tremor and regurgitation. Unexpectedly, the following morning the seal was found dead outside its pool. Necropsy of the carcass did not reveal obvious macroscopic pathological changes. However, histopathology showed generalised mild to moderate lymphoplasmacytic infiltrations of brain and cervical spinal cord associated with neuronal destruction and mild meningitis. These findings, clinical history and geographic location prompted the suspicion of WNV infection. Subsequent nucleic acid molecular investigations by RTqPCR of various organ tissues indeed confirmed WNV infection in this animal, while avian influenza virus was not found.

As WNV infection can lead to fatal neuroinvasive disease, in regions of autochthone infections WNV should be considered as a possible causative agent in neurologically conspicuous animal or human patients.

Keywords:

West Nile Virus, emerging diseases, wildlife, pathology, virology

Lunch & Poster Viewing / Abstract-ID 293

Tick-borne encephalitis virus in ticks from Latvia

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Ticks are important parasites of economic and public health due to their ability to transmit zoonotic diseases. Tick-borne encephalitis virus (TBEV) is a Flavivirus with five main subtypes of which three, the European (TBEV-EU), the Siberian (TBEV-Sib) and the Far Eastern subtypes (TBEV-FE) are supposed to circulate in Latvia. Several hard tick species are involved in TBEV circulation and transmission in nature. In Latvia, only few studies concerning TBEV circulation have been conducted and knowledge about the distribution of TBEV subtypes is at best fragmentary.

The aim of the present study was detecting and characterizing the TBEV subtypes circulating in Latvian ticks. In 2019 and 2021 to 2023, ticks were collected by flagging in two Latvian regions. Ticks were morphologically identified and pooled (10 ticks/pool) and screened for TBEV RNA using a RT-qPCR. The positive pools were further investigated by sequencing the full genome and virus isolation. Totally, 2,421 ticks were collected, with *Ixodes ricinus* as the dominant species (2,287 specimens) followed by *Ixodes persulcatus* (130 specimens) and *Dermacentor reticulatus* (4 specimens). *Ixodes ricinus* carried TBEV-EU and TBEV-Sib, while *I. persulcatus* carried only TBEV-Sib. In conclusion, two TBEV subtypes were detected and isolated in Latvia. Further investigations are necessary to better understand the natural transmission and the medical importance of these TBEV.

Keywords:

Tick-borne encephalitis, ticks, Latvia

Lunch & Poster Viewing / Abstract-ID 300

Geographic distribution of animal aggressions in the Selva Maya region of Mexico

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The Selva Maya region and its protected areas (PAs) in Mexico face threats from ecosystem fragmentation and human encroachment, creating opportunities for zoonotic spillovers. An increase in wildlife-transmitted rabies has been observed in livestock, domestic animals, and humans, due to bites from bats, skunks, and cats. This study aimed to define the geographic distribution of animal aggression events towards humans as an indicator of rabies transmission risk and to identify sociodemographic and environmental factors potentially associated with these events. Animal aggressions in the region were obtained based on the geographic location of the reporting health units. Sociodemographic variables at the Basic Geostatistical Area (AGEB) level were linked to the health units using an estimated catchment area defined by the shortest travel distance. The catchment area is also used to determine exposure to indicators of biodiversity changes. Preliminary analyses employing a hierarchical, zero-inflated Poisson regression model suggested that animal aggressions tended to concentrate in more populated areas but also occurred near PAs. Localities with farming as the primary economic activity had a higher probability of animal aggressions than those with agriculture, while ranches or farms were more prone to aggression events than villages. These findings allow further developments of the model to inform targeted risk communication strategies to prevent rabies transmission.

Keywords:

wildlife, rabies, Selva Maya, zoonosis, One health



Commensal rodent pathogens

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Rodents are the most speciose mammalian group, encompassing some ~2300 species. This great diversity is associated with a correspondingly significant pathogen diversity, including Seoul virus, lymphocytic choriomeningitis virus (LCMV), rat hepatitis E virus, *Leptospira* spp. and *Streptobacillus moniliformis*. Commensal rodent species such as Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) are particularly important pathogen reservoirs because of their close association with humans. Here, we present the results from several rodent pathogen studies from Europe and the Middle East. We describe the prevalence and diversity of rodent-borne pathogens in urban areas, and present case studies from zoos in Germany. In Iran we detected the presence of eight different pathogens, several of which being zoonotic, and multiple coinfections. We were also able to detect several mutations of the *Vkorc1* gene in these rats, which is responsible for resistance to anticoagulant rodenticides. In German zoos we describe the potential spill-over of *Francisella tularensis* and LCMV from wild rodents to captive monkeys. Furthermore, we describe the re-emergence and persistence of LCMV in a mouse population in Germany and the potential for spill-over events in zoos. These studies highlight the need for continued pest rodent management and surveillance, and the potential risk these animals pose for the emergence and re-emergence of zoonotic pathogens.

Keywords:

rodents, ecology, LCMV, rat hepatitis E virus, Germany, zoos

Lunch & Poster Viewing / Abstract-ID 305

Taenia martis in a white-headed lemur (*Eulemur albifrons*) from a Zoological Park in North Rhine-Westphalia, Germany.

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Taenia(*T.*) *martis* is a zoonotic tapeworm found in the small intestine of carnivores, mostly of the *Martes* genus. In zoos, *T. martis* eggs can find a comfortable shortcut to new (and old) hosts. We report an example of this in a white-headed lemur, which was found dead in a zoological park.

A 5-year-old female white-headed lemur without previous history of disease was unexpectedly found dead in her enclosure in a zoological park, where she lived in a group of five lemurs. A complete necropsy was performed and samples were taken for histological examination and DNA purification. After DNA purification, a PCR for the amplification of the 12S rRNA mitochondrial fragment was performed. Subsequently, the amplicons were sequenced using Oxford nanopore next-generation-sequencing techniques. A cysticercal larva was found inside a nodule adherent to the pleura. Sequences of the PCR amplicons identified the larva as *T. martis*.

This is the first report of *T. martis* infection in a white-headed lemur. The origin of the infection was not determined but direct contamination with *Taenia* from the vicinity or food contamination are possible. *T. martis* is a zoonotic parasite. Therefore, animal keepers, veterinarians and other zoo staff are at risk for infection. Finally, *T. martis* infection could be fatal in non-human primate species which makes this case report also relevant with regard to conservation efforts.

Keywords:

Taenia martis, zoonoses, wildlife, one health

Lunch & Poster Viewing / Abstract-ID 310

Puumala orthohantavirus monitoring in bank voles to assess the epidemiological situation in Germany

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Puumala orthohantavirus (PUUV) causes more than 80% of human hantavirus disease cases in Germany. The incidence of human cases depends on the abundance of the natural reservoir, the bank vole (*Myodes glareolus*) and its PUUV prevalence. The objective of our study within the research consortium “RoBoPub” (Rodent-Borne-Pathogens-and-Public-Health) is a further characterization of the PUUV range in Germany. For that purpose, bank voles were trapped along transects in North Rhine-Westphalia/Lower Saxony, within Thuringia and Bavaria.

Between 2018-2021 3,320 bank voles were collected and screened for PUUV-specific RNA and antibodies. While in spring the average PUUV prevalence was low to moderate (16%) in non-outbreak years, high values were reached (up to 92%) in outbreak years 2019 and 2021. The investigation in Lower Saxony confirmed the distribution border reaching from district Grafschaft Bentheim to district Osnabrück in the northwest of Germany. In Thuringia, PUUV occurrence in bank voles was rare and strongly restricted to certain areas in forests of the northwest and southwest. Data from Bavaria suggest the absence of PUUV within the central region despite its widespread presence in Bavaria's north and east. Future investigations have to identify reasons for the heterogeneous PUUV distribution in Thuringia and Bavaria. The identification of PUUV endemic regions based on reservoir screening, will help to establish geographically specified risk assessments for the public.

Keywords:

Puumala orthohantavirus, bank vole, reservoir, prevalence



Prion Protein Gene Diversity in German, Danish and Polish deer species

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Genetic variability of the Prion Protein Gene (PRNP) influences disease susceptibility of certain Transmissible spongiform encephalopathies, incl. Chronic Wasting Disease (CWD) in cervids. We therefore analysed PRNP genotypes in German and Danish red deer (*Cervus elaphus*), German roe (*Capreolus capreolus*) and sika deer (*Cervus nippon*) and Polish moose (*Alces alces*) by sequencing the open reading frame on exon three of the PRNP. Samples were submitted by German hunters, foresters and pathologists and from the Danish Frøslev Plantage, Polish moose samples are of the CWD monitoring program. All roe deer (297/297) and (81/81) moose were homozygous for wildtype TPQ, with a silent mutation (SM) at codon 42 in roe deer (5/297) and codon 20 in moose (11/81). Genetic variability was seen in red and sika deer. Red deer showed two non-synonymous polymorphisms (PM) at codons 98 and 226, a 24bp deletion and three SM at codons 21, 78 and 136. In summary six genotypes were detected: homozygote TPQ (50/278), TPE (116/278), APQ (9/278) and heterozygote TPQ/TPE (44/278), TPQ/APQ (20/278) and TPE/APQ (39/278). Sika deer revealed TPQ (36/40), TPQ/TPE (3/40) and TPE/APQ (1/40) as well as SMs at codons 133 (4/40) and 136 (4/40), the former being sika specific. The influence of the described genotypes on CWD-susceptibility remains to be tested, however these results extend the knowledge of European cervid genotypes and help adjusting the EU CWD surveillance and control measures.

Keywords:

Chronic Wasting Disease, Cervids, deer, Prion, PRNP, genotype

Lunch & Poster Viewing / Abstract-ID 316

Characterization of Shigatoxin-producing Escherichia coli isolated from food harbouring the rare Shigatoxin subtype stx2i

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Shigatoxin-producing Escherichia coli (STEC) are important food-borne pathogens. There are several Shigatoxin (Stx) subtypes known (Stx1a, c, d, Stx2a-o). These vary in their prevalence but can also vary in their toxic effect. For the subtype Stx2i only two isolates have been published until now. In 2020 and 2021 two further isolates have been identified as stx2i gene positive. These strains were isolated from lamp meat in Germany and were characterized concerning serotype and virulence associated genes by classical serological methods, PCR and whole genome sequencing (WGS).

Using classical methods for the two strains serotypes ONT:H21 and ONT:H25 were determined, respectively. WGS revealed genoserotypes Onovel8:H21 and O8/O30:H25. Real time PCRs according to ISO13136 and Pavlovic et al. were able to detect stx genes in both strains; however, PCR methods used for routine subtyping of the stx gene showed inconsistent results for stx2a, stx2c/d and stx2g. Stx gene sequences determined from WGS showed a 100 % identity to a previous published strain from Norway. Toxin production was proven by an enzyme linked immunosorbent assay. Both strains were negative for virulence genes like eae, ehxA und nleB.

WGS analyses are an important method for the determination of those Shigatoxin variants. The determination of the pathogenic potential of the new Stx2i strains is challenging as few is known about STEC with the Stx2i subtype and its occurrence in natural hosts and the environment.

Keywords:

STEC, food-borne pathogen



Lunch & Poster Viewing / Abstract-ID 324

Molecular characterization of *Streptococcus pneumoniae* strains circulating at the human-wildlife interface in tropical sub-Saharan Africa

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Infections with *Streptococcus pneumoniae* pose a major threat to wild great apes and humans, especially in developing countries, where limited surveillance results in a lack of data on circulating serotypes, virulence and resistance genes, which is needed for effective prevention and response measures.

Our study characterizes pneumococcal strains in humans and wild great apes in two remote African regions: the Taï National Park (Côte d'Ivoire) and the Dzangha-Sangha Protected Areas (Central African Republic). Sampling takes place during regular staff health checks for humans working in proximity to wildlife and non-invasive methods (e.g. fruit swabs and feces) for great apes. Pneumococcal carriage strains were isolated from the specimens directly in the field using a mobile, solar-powered incubator. Molecular analyses including PCR and next-generation sequencing are used for genomic characterization.

During the first field missions, 216 human nasopharyngeal swabs and 75 fruit swabs of Western chimpanzees (*Pan troglodytes verus*) were collected. In total, 45 α -hemolytic colonies from human specimens and 96 from chimpanzee specimens were isolated. Of the isolates from human specimens, at least 14 were confirmed as *S. pneumoniae*. The remaining 96 isolates are still being characterized. These preliminary results suggest that cultivating directly in the field is a suitable method to obtain pneumococcal isolates to characterize bacterial transmission at the human-great ape interface.

Keywords:

pneumococcal carriage, mobile incubator, human-great ape interface

Lunch & Poster Viewing / Abstract-ID 241

Development of an artificial intelligence-based workflow for the reliable identification of mosquito species by non-expert user groups

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Globalization and climate change drive the global spread of invasive mosquitoes and pathogens, increasing the risk of mosquito-borne disease outbreaks in previously unaffected regions. Therefore, accurate identification of mosquito species and robust vector surveillance are crucial to allow appropriate response, e.g., control measurements. However, they often necessitate advanced training and costly laboratory equipment. Previous studies have demonstrated the exceptional pattern recognition capabilities of Convolutional Neural Networks (CNNs), enabling accurate, automatic, and quick classification of mosquitoes based on morphological features. Our research compared the performance of CNNs using smartphone and microscope images of mosquito bodies and wings. The results revealed comparable accuracy between both types of images capturing (~90%), with CNNs achieving higher accuracy when using wing images (+ 8%). Building upon these findings, our aim is to develop a robust classification algorithm using CNNs deployed to smartphone images to support vector surveillance efforts, particularly in resource-limited settings. Our approach addresses model uncertainty, enabling the CNNs to detect instances of uncertainty in their predictions or recognition of unfamiliar species. Implementation of this algorithm can significantly improve vector surveillance in settings where access to trained entomologists and advanced laboratory equipment is limited.

Keywords:

AI, Computer Vision, Vector Surveillance

Lunch & Poster Viewing / Abstract-ID 308

Comparison of Illumina and Oxford Nanopore Technology for genome analysis of *Francisella tularensis*, *Bacillus anthracis*, and *Brucella suis*

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Bacterial epidemiology requires understanding the spread of highly pathogenic bacteria like *Bacillus anthracis*, *Brucella* species, and *Francisella tularensis*. Whole genome sequencing (WGS) and genotyping are crucial for this purpose. While Illumina short-read sequencing is established, the suitability of Oxford Nanopore Technology (ONT) long-read sequencing remains unexplored.

This study evaluated Illumina, ONT flow cell versions 9.4.1 and 10.4 for six strains respectively, for each selected species. ONT produced ultra-long reads, while Illumina provided short reads with high accuracy. Flow cell version 10.4 improved accuracy. All technologies accurately identified the species and virulence markers. ONT allowed near closure assembly of chromosomes and virulence plasmids for *Ba. anthracis*.

All assemblies based on ONT, Illumina, and hybrid approaches correctly predicted canonical (sub-)clades for *Ba. anthracis* and *F. tularensis* and Multi-Locus Sequence Types (STs) for *Br. suis*. High-resolution genotyping showed comparable results between Illumina and ONT data for *F. tularensis*. *Ba. anthracis* aligned well only with flow cell version 10.4. *Br. suis* showed larger differences between Illumina and ONT.

In conclusion, combining ONT and Illumina data is feasible for high-resolution genotyping in *F. tularensis* and *Ba. anthracis*, but not yet for *Br. suis*. Improvements in nanopore technology may enable genotyping for all bacteria in the future.

Keywords:

Bacillus anthracis; *Brucella*; *Francisella tularensis*; Genome sequencing; Illumina; Oxford nanopore technology; R10.

Young Scientist Breakfast

Requirements

- Young Scientist (doctoral student or postdoc < 3 years post-graduation)

AND

- member of the Zoonoses Platform

When

October 10th, 2023 from 9:00 to 10:30 am

Where

MOA Eat (restaurant of the event's venue site)

During “Zoonoses 2023 – International Symposium on Zoonoses Research” young scientists, who are members of the Zoonoses Platform, have the unique opportunity to meet distinguished scientists from the community at an informal joint breakfast. While you are enjoying your breakfast at the restaurant of the event's venue site (MOA Eat), you may ask senior scientists about their careers and get valuable tips for your own career in science. The Young Scientist Breakfast takes place on October 11th, 2023 from 9:00 to 10:30 am. In order to be able to take advantage of this opportunity, it is necessary that you state your interest in participating in the Young Scientist Breakfast. Please note that the number of places is limited. We will inform you a few weeks before the event if you have a place. If you are not able to take your place, please let us know in time so that we can allocate the place to someone else.

Senior Scientists

Prefix / Title	First Name	Last Name	E-mail address	Institute / Company
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Participants 2023

Prefix / Title	First Name	Last Name	City
Dr	Prerna	Arora	Göttingen
Dr	Aileen	Faist	Münster
	Christin	Körsten	Greifswald - Insel Riems
Dr	Jasmin	Wenderlein	Oberschleißheim
Mr	Mauricio Alejandro	Andino Molina	Jena
Ms	Faiza	Asghar	Erlangen
Ms	Aleksandra	Atanasova	Potsdam
	Jana	Brendecke	Braunschweig
Mrs	Sabrina	Clever	Hannover
Mrs	Annika	Dohme	Berlin
Ms	Annika	Fischer	Berlin
Ms	Sarah	Gothe	Hamburg
Mrs	Harriet	Herridge	Greifswald
Ms	Jessica	Panajotov	Berlin
Ms	Thalia	Preuß	Berlin
	Nunzio	Sarnino	Berlin
Ms	Lu	Zhang	goettingen
Ms	Ann-Kathrin	Brüggemann	Essen
	Ana	Carranco	Ulm
Mrs	Julia	Finkensieper	Leipzig

Lessons Learned from the Old and New World: Exploring the Interplay between Biodiversity and Zoonotic Diseases in Wildlife

Authors: Magdalena Meyer¹; Dominik Schmid; Georg Eibner; Kerstin Wilhelm; Heather J. Baldwin; Ramona Fleischer; Alexander Heni; Evans Ewald Nkrumah; Ebenezer K. Badu; Samuel Kingsley Opong; Nina Schwensow; Peter Vallo; Victor Corman; Marco Tschapka; Christian Drosten; Simone Sommer

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Understanding the relationships between biodiversity and zoonotic diseases is crucial for safeguarding human and animal health. We investigated the diversity-disease relationships using bat communities exposed to coronaviruses (Ghana) and placental and marsupial mammal communities exposed to Trypanosoma infections (Panama) as study systems. Using spatiotemporal variations in species community assemblages, we revealed in both settings and study systems that anthropogenic disturbances and subsequent biodiversity loss are reshaping species communities, favoring the proliferation of generalist species that act as main reservoirs for pathogens. As a consequence, infection probability and prevalence increased in less diverse species assemblages that are dominated by susceptible hosts. In Ghanaian bats, we were able to distinguish between taxa that amplify or reduce coronavirus infection likelihood. In Panama, we found that Trypanosoma infection likelihood was primarily associated with marsupial density in relation to human disturbance. Combined, both host-pathogen systems highlight aspects of diversity-disease relationships compatible with the dilution effect hypothesis. Overall, our findings emphasize the urgent need to prioritize conservation efforts that maintain healthy and resilient ecosystems to mitigate the risks associated with zoonotic disease transmission.

Keywords:

species diversity, host community composition, dilution effect, anthropogenic disturbance, coronavirus, Trypanosoma, Ghana, Panama

Session 9: Zoonoses & Wildlife I / Abstract-ID 200

Linking immunogenetics to tuberculosis susceptibility and progression in wild meerkats

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Tuberculosis (TB) remains a major cause of morbidity and mortality in humans and livestock, with high zoonotic and epizootic potential based on the high transmissibility between species. Host-Myco-bacterium interactions are complex, and despite highly infectious clinical stages, only a fraction of infected hosts contribute to TB transmission. In wildlife, general predictors of TB progression and disease dynamics are poorly understood. Over the last two decades, wildlife TB has been on the rise in Southern Africa, affecting also the wild meerkats (*Suricata suricatta*) intensely studied within the Kalahari Meerkat Project. Infection prevalence with *M. suricattae* has increased since the late 1990s, contributing to meerkat mortality. Despite high exposure levels, there is marked variation in TB progression, with many individuals never displaying overt signs of TB. Here, we capitalize on the exceptional long-term dataset of life-history and health records to investigate the immune-genetic basis of variation in TB progression. More than 1500 individuals alive between 1999 and 2023 were genotyped at the major histocompatibility complex class II DRB-exon 2 locus to investigate whether MHC composition and/ or functional diversity contribute to TB susceptibility, progression and survival. This project advances our understanding of the role of genetics in TB epidemiology, potentially allowing for extrapolation of the findings to other, less well studied mammal species affected by TB.

Keywords:

Tuberculosis, Major histocompatibility complex, long-term study, meerkats (*Suricata suricatta*), disease transmission

Session 9: Zoonoses & Wildlife I / Abstract-ID 313

Small but powerful - decoding the virome of selected crocidurine shrew species

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Knowledge on potential pathogens in wildlife reservoirs is important for pandemic preparedness. Shrews are a large and phylogenetically old species group, but their virome still remains elusive. We investigated four shrew species (bicolored, greater and lesser white-toothed shrews, Etruscan shrews) from Europe with a metagenomics next-generation sequencing approach.

RNA was extracted from 54 individual based organ pools. After rRNA removal, cDNA libraries were generated and sequenced with Illumina technology (~ 1.5 billion reads in total). The *de novo* assembled transcripts were analysed and taxonomically classified. Additionally, RT-qPCRs were designed for newly detected viral sequences.

Whole-genome sequences were obtained for several novel viruses of the families *Nairoviridae*, *Paramyxoviridae* and *Hepeviridae*. A high prevalence of genetically diverse orthonairoviruses was detected, which are genetically related to viruses of the Crimean-Congo haemorrhagic fever virus group. Furthermore, one of at least three novel orthoparamyxoviruses is closely related to the zoonotic *Langya virus* from China. And they form a phylogenetic sister group to the bat-derived henipaviruses.

Overall, our study is the first to shed light on the virosphere of four different crocidurine shrew species from Europe and revealed a number of novel interesting viruses. However, further investigations are needed to characterize these novel viruses and to assess their zoonotic potential.

Keywords:

metagenomics, virome, white-toothed shrews, paramyxovirus, nairovirus, hepevirus

Session 9: Zoonoses & Wildlife I / Abstract-ID 307

Unraveling the Molecular Evolution of Usutu Virus in Germany using Nanopore Sequencing

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In Germany, the mosquito-borne flavivirus Usutu virus (USUV) is the causative agent of annually re-occurring epizootics in the avifauna, primarily of blackbirds and owls. USUV was first documented in 2010 in the southwest of Germany in *Culex* mosquitoes and in 2011 in blackbirds. Ongoing surveillance efforts identified a spread of the virus to the north and east of the country, upon where it reached all Federal States in 2018. The same year in which the largest recorded epizootic occurred in the country (1,208 USUV-positive birds). Despite its relevance for not only veterinary but also public health, little research in the past has focused on gathering USUV whole genome sequences and analyzing the virus's molecular evolution. Therefore, in the frame of this study, a Nanopore sequencing tool was implemented for USUV and compared to that of Illumina sequencing as well as to a protocol with and without prior target-enrichment. The subsequent cost- and time-effective amplicon-based Nanopore platform was then used to generate 118 USUV whole genome sequences from wild and captive birds from Germany. Phylogenetic and phylodynamic analyses revealed the prevalence of lineages Europe 3 and Africa 3 in Germany and that their most-recent common ancestors confirm a 3-year time-lag between their possible introduction and their actual identification. Furthermore, the study provides indices for the genetic evolution of USUV within the country as well as frequent new introductions thereof.

Keywords:

Germany, Nanopore, Surveillance, Usutu virus, Whole genome sequencing

Session 9: Zoonoses & Wildlife I / Abstract-ID 287

Viral interaction between West Nile virus and Usutu virus during simultaneous co-infections in frequent German mosquito species

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The two closely related flaviviruses West Nile virus (WNV) and Usutu virus (USUV) co-circulate in many European countries including Germany. Both viruses circulate in an enzootic cycle between birds and mosquitoes as biological vectors. In addition, transmission to vertebrate species such as horses and humans is possible. Infections can lead to serious illnesses, and WNV in particular poses a risk to the safety of blood donations.

Co-infections with WNV and USUV in hosts and vectors could have unexpected consequences for their transmission, however, these potential impacts are to date mostly unknown. Therefore, simultaneous co-infections in the mosquito species *Culex pipiens* biotype *pipiens* (CxP), *Culex pipiens* biotype *molestus* (CxM) and *Aedes vexans* (AeV) were performed.

The results showed that both viruses appear to interact during replication in the mosquito. This interaction led to a reduction in USUV susceptibility in CxP but to an increase in USUV susceptibility in AeV. In contrast, no mutual influence between WNV and USUV could be detected in CxM. In addition, potential co-transmission of both viruses was detected in CxM.

Thus, it could be shown that even a non-vector-competent species (AeV) might play a role in transmission of WNV and USUV in the presence of co-infections. In addition, the outcome of viral interaction seems to vary between different mosquito species. These results should be included in future WNV and USUV surveillance strategies in Germany.

Keywords:

Culex pipiens, *Aedes vexans*, West Nile virus, Usutu virus, co-infections

Session 9: Zoonoses & Wildlife I / Abstract-ID 222

Proximity loggers to study rodent spatial behaviour and pathogen transfer in farming environments

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In farming, rodents cause crop loss, infrastructure damage and have a high potential to transmit pathogens to humans and livestock. Diseases related to the latter are encephalomyocarditis, leptospirosis and porcine circovirus that can all result in high economic losses. Pathogen transfer could be dependent on rodent density and favoured by frequent contacts between rodents, livestock and livestock feed. It is therefore important to understand the rodent movement patterns on farms as well as contacts among them, with livestock and livestock holding elements. This could help to develop strategies to minimise damage and health risk to livestock and farm staff. In the EU-funded Rodentgate project, we aim to study these patterns in Norway rats (*Rattus norvegicus*) on farms. Miniaturised proximity loggers are newly developed tools for monitoring the spatial behaviour and contacts by using Bluetooth signal strength as an indicator of contacts between individuals and of individuals with relevant structures. Results from dry runs to test and calibrate this novel technology provide initial experience with this methodology in an agricultural setting and will be presented. These tests yield information about the impact of physical barriers that can interfere with Bluetooth signal. By trapping, tagging and recapturing wild rodents, we aim to record contact rates to identify potential transmission hotspots and contribute to a better understanding of rodent pathogen transmission on farms.

Keywords:

Rodents; Contacts; Disease transmission; Livestock; Movement patterns

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 233

From Bits to Insights: Leveraging Multidisciplinary Data for Modern Epidemiological Surveillance and Risk Assessment

Authors: Juliane Boenecke¹; Jonathan Ströbele²; Kristopher Nolte²; Ulfia A. Lenfers²; Nima Ahmady-Moghaddam²; Matthias H. Belau³; Mirko Himmel⁴; Amena Almes Ahmad²; Ummul-Khair Mustafa⁵; Luba Pascoe⁵; Daria Dretvić⁴; Katharina S. Kreppel⁵; Elingarami Sauli⁵; Devotha G. Nyambo⁵; Jennifer L. Pohlmann²; Walter Leal Filho²; Wolfgang Streit⁴; Heiko Becher⁶; Ralf Reintjes²; Johanna Brinkel¹; Jürgen May¹; Thomas Clemen²

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Digitalization is changing the landscape of infectious disease research. It not only enhances data collection and processing but brings together data, data-related resources, and diverse stakeholders, facilitating interdisciplinary collaboration. Building on the experiences of the international research network ESIDA (Epidemiological Surveillance of Infectious Diseases in Sub-Saharan Africa), we showcase the practices of a collaborative Data Ecosystem that aims to harness digital data and computational methods to strengthen epidemiological surveillance. Its central feature is a novel spatio-temporal information system that harmonizes outbreak signals and versatile socio-ecological context data from open sources to map epidemic events and provide actionable information on associated risks, impacts, and trajectories at different sub-national levels. The dengue virus, which proliferates globally, serves as our test case, with a focus on LMICs (Tanzania). Through the integration of 103 data layers across five categories (socio-demographic, environmental, meteorological, health, and infrastructure), we outline potential applications in modern surveillance and risk assessment, as well as peculiarities in handling data from various domains. With data collaboration being an ideal entry point for interdisciplinary exchange, we aim to explore further new data and research interactions within the zoonoses research community, e.g., from viral ecology or data-driven risk modeling.

Keywords:

Digital Advances; Data Collaboration; Epidemiological Surveillance; Risk Assessment

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 198

Compatibility of WGS data from Illumina and Ion Torrent technology in genome comparison analysis of *Listeria monocytogenes*

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Whole genome sequencing (WGS) has become the key approach for molecular surveillance of the food-borne pathogen *Listeria monocytogenes* (Lm). Genome comparison analysis can reveal transmission routes that cannot be found with classic epidemiology.

Widespread standard for use in genome comparison analysis is data from short read sequencing, generated on Illumina or Ion Torrent devices. To date, little is known on the compatibility of data from both platforms. This knowledge is essential when it comes to the central analysis of data, e.g. in the case of outbreaks.

We used WGS data from 47 Lm isolates of the strain collection of the National Reference Laboratory for Lm, generated on either Illumina or Ion Torrent devices, to analyse the impact of the sequencing technology on downstream analyses. In our study, only the assembler SPAdes delivered qualitatively comparable results. In the gene-based core genome multi locus sequence typing (cgMLST), the same-strain allele discrepancy between the platforms was 14.5 alleles on average, which is well above the threshold of 7 alleles routinely used for cluster detection in Lm. Application of a strict frameshift filter could push the mean discrepancy below this threshold, but reduced discriminatory power. The impact of the platform on the read-based single nucleotide polymorphism (SNP) analysis was lower than on the cgMLST. Overall, it was possible to improve compatibility in various ways, but perfect compatibility could not be achieved.

Keywords:

Whole Genome Sequencing, Genome Comparison, cgMLST, SNP, Illumina, Ion Torrent

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 191

Development of an efficient reverse genetics system for HEV genotype 1 for use in comparative studies with zoonotic genotype 3

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Hepatitis E virus (HEV) genotype 1 (gt1) infects only humans whereas genotype 3 (gt3) is zoonotic infecting various animal species and humans. For elucidation of determinants of host specificity, experiments using reverse genetics enabling targeted genome manipulations are needed. However, whereas a few reverse genetics systems exist for gt3, those for gt1 are still very limited, mainly because of inefficient replication of this genotype in cell cultures. Here, the generation of gt1 strain Sar55 and gt3 strain 47832mc by transfecting in vitro transcribed and capped virus genomes into different cell lines was attempted. Transfection of the human hepatoma cell lines PLC/PRF/5 and HuH7-Lunet-BLR resulted in increasing amounts of viral RNA and protein for both genotypes; however, significant numbers of infectious particles were only present in case of gt3. In contrast, transfecting the intestinal cell line Caco2 resulted in the release of infectious gt1 and, to a lesser extent, gt3 into the supernatant. Gt1 reached titers >1000 infectious particles/ml and could be readily passaged on fresh Caco2 cells. Density gradient analyses indicated that the majority of gt1 virus particles were enveloped in analogy to gt3. In contrast, less secreted capsid protein was present in case of gt1 compared to gt3. The results indicate that gt1 is able to exert a complete replication cycle in Caco2 cells and provide an efficient reverse genetics system for this genotype for future studies.

Keywords:

Hepatitis E virus, cell culture, replication, transfection

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 235

Genomic analysis of *Streptococcus suis* isolated from healthy and diseased birds

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Streptococcus suis is a respiratory commensal of pigs, with some lineages causing serious swine disease and zoonotic disease in humans. In recent years, *S. suis* has also been isolated from cats, dogs, cattle, sheep, wild boars, and different bird species, including chicken. It is generally assumed that, as in humans, these infections are due to “spillovers” from pigs, but no genomic investigation of respective isolates has yet been performed. Over the last six years, we collected and whole-genome sequenced *S. suis* samples from birds in the veterinary diagnostics department of the Freie Universität Berlin. Most of these birds were diseased and *S. suis* was isolated as the causative pathogenic agent. We combined these samples with published genomes of Vietnamese chicken isolates and compared them to *S. suis* lineages isolated from pigs and wild boars. All the bird isolates clustered phylogenetically within a group of largely commensal isolates of pigs, and distinct from the lineage responsible for most zoonoses. Bird isolates also lacked most known virulence genes but were often multi-drug resistant. We detected a significant overrepresentation of unique genomic islands within bird isolates, suggestive of *S. suis* adaptation to birds. Taken together, our results imply that *S. suis* could be persisting in bird populations independently of pigs, at least for short periods, but that birds are unlikely to be a source of zoonotic infection in humans.

Keywords:

Streptococcus suis, birds, sequencing, genome analysis

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 319

Phenotypic and genotypic antimicrobial resistance of *Streptococcus uberis* isolated from bovine mastitis in Ruringia, Germany

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Bovine mastitis is a highly prevalent disease plaguing the dairy industry. *Streptococcus uberis* is the most predominant pathogen for bovine mastitis and is frequently isolated from milk of infected quarters. This study aimed to identify the phenotypic and genotypic antimicrobial resistance (AMR). 80 *S. uberis* were isolated from milk samples of dairy cows with clinical mastitis in Thuringia. Phenotypic AMR testing was achieved for all strains against 24 clinically relevant antimicrobial agents using a broth microdilution test. Illumina Miseq was used for genomic sequencing of 80 strains and 24 strains were further sequenced by Oxford Nanopore Technologies' MinIon. Bioinformatic analysis for AMR genes, plasmids, MLST, and virulence associated genes was performed. Phenotypic AMR against 5 antibiotic classes (β -lactams, lincosamides, aminoglycosides, macrolides, fluoroquinolones) were determined. Genetic marker for resistance against tetracyclines, lincosamides, aminoglycosides, and macrolides were detected. 21% of the strains were resistant against pirlimycin (lincosamides), while *tet(M)* associated with tetracycline resistance was the commonest detected genetic marker. 93% of the sequenced strains contain plasmid-borne sequences indicating a possible transmission of AMR genes. While the genomes of *S. uberis* generally show a high diversity, indicated by 57 detected sequence types, a few genomically highly similar strains persisted within farms or circulated between farms.

Keywords:

Bovine mastitis; *S. uberis*; genome sequencing; AMR; virulence; MinIon; Illumina;

Session 10: Bioinformatics, Digitalization and AI in One Health Research / Abstract-ID 329

Towards reliable prediction of significant changes in microbial communities based on time series data

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Changes in the microbiome can be an indicator of the onset of an infectious disease such as sepsis. The ability to distinguish these significant changes from naturally occurring fluctuations in the microbiome could aid in the early detection of potentially harmful diseases. We aim to take a step toward this prediction of microbial abundance trends based on analysis of 16S rRNA data. To this end, we apply Long Short-Term Memory (LSTM) models to publicly available long-term microbial time series data from two healthy subjects. To increase the explanatory power of the model, Shapley Additive Explanations (SHAP) is used for feature significance analysis. So far, the model has shown good performance in predicting the overall abundance of bacterial genera in the samples from the healthy subjects. We plan to test different model architectures and extend the model to other data types such as environmental data. Our overall goal is to optimize early therapeutic approaches and provide a treatment advantage to physicians and patients on the one hand, and to create an environmental monitoring system on the other.

Keywords:

Machine learning

Session 11: Host-pathogen Interactions 2 / Abstract-ID 202

MHC genes regulate host gut microbiome in bats with acute coronavirus infections

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Genes of the exceptionally polymorphic Major Histocompatibility Complex (MHC) region play a critical role in pathogen defence and the cross-talk with host commensals. Yet, it remains unclear how the gut microbiota response to infection and to what extent this response is influenced by host immunogenetics. Here, we aimed to disentangle their interplay in roughly 600 African hipposiderid bats, which harbour the most recent coronavirus ancestors to HCoV-229E and SARS-like CoVs, both of which replicate enterically, where MHC class II expression is densest and gut microbiota flourishes. We previously linked host MHC diversity and certain MHC supertypes to CoV infections. Now we uncovered that gut microbial alpha-diversity differed between CoV infections, and microbial network structure varied decisively between uninfected, singly and co-infected bats with potentially pathogenic bacteria taking the role of hub taxa in CoV infected bats. Joint species distribution model underscores that several mutualistic and potentially pathogenic taxa differ between infection status. When host immunogenetic information in form of MHC diversity and supertypes was added, several interactions emerged in prominent bacterial genera. Our work suggests that host immune genes and CoV infection influence gut microbiota independently, but also highlights that their interaction shapes host susceptibility to CoV infections and the establishment and persistence of a pathobiome.

Keywords:

Microbiome, MHC, Coronavirus, Ghana, co-infections

Session 11: Host-pathogen Interactions 2 / Abstract-ID 190

The bat-derived influenza A viruses H18N11 infects and replicates in leukocytes

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All known conventional influenza A viruses (IAVs) circulating in birds, pigs and humans infect cells by binding to sialic acid receptors on host membrane glycoproteins. Natural infections usually affect the intestinal or respiratory epithelia and only exceptionally other cell types. In contrast, the bat-derived IAV H18N11 utilizes major histocompatibility complex class II (MHC-II) molecules for cell entry. We have previously found that H18N11 replicates in the tonsils and intestinal Peyer's patches of its reservoir species, the Jamaican fruit bat. However, it has been unclear whether viral replication is restricted to epithelial cells or whether other MHC-II-expressing cells of these lymphoid tissues are similarly susceptible to infection. To identify the cellular tropism of H18N11 in Jamaican fruit bats and determine the induced immune response to infection we performed single-cell RNA sequencing, immunohistochemistry and RNAscope. We show that H18N11 preferentially manifests infection in a range of leukocytes, including macrophages, B cells and NK/T cells and less frequently in intestinal epithelial cells. Furthermore, while infection with H18N11 leads to a moderate induction of interferon-stimulated genes, we observe no detectable expression of interferons and pro-inflammatory cytokines. We also determine the capacity of H18N11 to infect human leukocytes. Interestingly, H18N11 is able to infect myeloid and lymphoid cells, and replicates efficiently in human macrophages.

Keywords:

bat-derived H18N11, single cell RNA-Sequencing, bat infection model, leukocyte infection

Session 11: Host-pathogen Interactions 2 / Abstract-ID 187

Therapeutic Oral Application of Carvacrol Alleviates Acute Campylobacteriosis in Mice Harboring a Human Gut Microbiota

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Human *Campylobacter jejuni* infections are rising globally. Since antibiotics are usually not indicated in campylobacteriosis, antibiotic-independent intervention measures are desirable. The phenolic compound carvacrol constitutes a promising candidate molecule given its antimicrobial and immune-modulatory features. To test the disease-alleviating effects of carvacrol treatment in acute murine campylobacteriosis, IL-10^{-/-} mice harboring a human gut microbiota were perorally infected with *C. jejuni* and treated with carvacrol via the drinking water. Whereas *C. jejuni* stably established in the gastrointestinal tract of mice from the placebo cohort, carvacrol treatment resulted in lower pathogen loads in the small intestines on day 6 post infection. When compared to placebo treatment, carvacrol application ameliorated pathogen-induced symptoms including bloody diarrhea that was accompanied by less distinct histopathological and apoptotic cell responses in the colon. Furthermore, innate and adaptive immune cell numbers were lower in the colon of carvacrol- versus placebo-treated mice. Notably, carvacrol application dampened *C. jejuni*-induced secretion of pro-inflammatory mediators in intestinal, extra-intestinal and systemic organs to naive levels. In conclusion, our preclinical placebo-controlled intervention study provides evidence that oral carvacrol treatment constitutes a promising option to alleviate campylobacteriosis in the infected vertebrate host.

Keywords:

carvacrol; enteropathogenic infection; *Campylobacter jejuni*; immune-modulatory effects; human gut microbiota-associated IL-10^{-/-} mice; campylobacteriosis model; host-pathogen interactions; placebo-controlled preclinical intervention study; One Health concept

Session 11: Host-pathogen Interactions 2 / Abstract-ID 218

Differential autophagy and immune signaling in human and *Rhinolophus* bat cells during SARS-CoV-2 infection

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Autophagy disruption can lead to autophagosome (AP) accumulation and SEC22B-mediated secretion of cytokine-carrying APs facilitating inflammation. SARS-CoV-2, which likely originated in *Rhinolophus* bats, blocks early innate immune activation and AP-lysosome fusion in human cells. Here, we explored whether reservoir and human host cells have differential immune and autophagy responses to SARS-CoV-2 infection.

Using live-cell imaging and a recombinant GFP-expressing SARS-CoV-2, we observed that human ACE2 transgenic *Rhinolophus ferrumequinum* lung cells (RhiFLu-hACE2) enable faster SARS-CoV-2 replication kinetics compared to human A549-hACE2. Intriguingly, IFN β and IFN ϵ mRNA levels were 75-fold higher in bat versus human cells at 2 dpi, indicating a rapid and strong immune induction in bat cells. SARS-CoV-2 infection reduced amount of lysosomes and disrupted AP-lysosome fusion in A549-hACE2 cells. In A549-hACE2 cells with LC3B and SEC22B knockout we confirmed autophagy-dependent secretion of IFN- α 2, IL-8, IL-18 and FGF-2 upon SARS-CoV-2 infection. Contrary, lysosome integrity and AP-lysosome fusion were unaltered in SARS-CoV-2-infected RhiFLu-hACE2 cells, likely due to an observed higher galectin-3 baseline expression, a protein involved in lysosome repair. In conclusion, our results suggest that SARS-CoV-2-infected reservoir RhiFLu-hACE2 cells rapidly activate innate immune responses, maintain autophagic flux with possibly limited secretory autophagy-mediated proinflammation.

Keywords:

Autophagy, secretory autophagy, cytokine secretion, SARS-CoV-2, bat immunology, innate immunity

Session 11: Host-pathogen Interactions 2 / Abstract-ID 219

Zapnometinib – A MEK1/2-inhibitor with broad anti-SARS-CoV-2 activity, synergistic potential and a reduced risk of resistance introduction

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Infections with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause coronavirus disease 2019 (COVID-19) with devastating consequences especially for high-risk patients. We could show that SARS-CoV-2 transiently activates the Raf/MEK/ERK pathway in the very early phase of the infection. Specific inhibition of the MEK1/2 kinases with the inhibitor ATR-002, now designated as Zapnometinib (ZMN), in single treatment scenarios as well as in combination with direct-acting antiviral drugs (DAAs), such as the nucleoside inhibitors Remdesivir, Molnupiravir, and the 3C-like protease inhibitors Nirmatrelvir and Ritonavir (Paxlovid), led to significantly reduced SARS-CoV-2 titers in different cell culture models and showed synergistic effects during the combinatory treatment. Additionally, ZMN diminished SARS-CoV-2-induced expression of pro-inflammatory cytokines, which is associated with hyperinflammation during COVID-19 progression. Serial passaging of the virus in the presents of ZMN or DAAs to force reduced drug susceptibilities showed no effect on ZMN passaged viruses, while the DAAs forced the virus to develop resistance introducing mutations. These data suggested the Raf/MEK/ERK signaling cascade as a druggable target for an anti-SARS-CoV-2 therapy and ZMN as a suitable drug for single and combinational treatment strategies with a very low risk of resistances.

Keywords:

Raf/MEK/ERK; SARS-CoV-2; Zapnometinib; Drug synergy; Drug resistance

Session 11: Host-pathogen Interactions 2 / Abstract-ID 224

Mosquito-specific viruses interfere with Culex-borne arboviruses

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Mosquito-specific viruses (MSVs) exclusively replicate within mosquitoes and can disrupt arbovirus infections by persistently infecting mosquitoes throughout their lifespan or multiple generations. MSVs exhibit a widespread presence in mosquito populations, including Germany, yet their impact on Culex-borne arboviruses currently circulating in Europe remains largely unexplored.

First, we determined the susceptibility of the “recently” created *Culex pipiens* cell lines for different MSVs and arboviruses. Secondly, we conducted co-infections of different *Culex*-derived cells with various MSVs and arboviruses from different families. In summary we found that the infection phase (acute or persistent) of the MSVs greatly affected the rate of arbovirus infections. A consistent negative effect was observed on both Semliki Forest virus and Bunyamwera orthobunya virus when using Eilat virus as an MSV; with the persistent phase demonstrating the most pronounced impact on this different arbovirus replication.

By studying the effects of different MSVs, this research enriches our understanding of MSV-mediated interference and its potential implications in combating mosquito-borne arboviruses.

Keywords:

Mosquito-specific virus, culex-borne arbovirus, BUNV, SFV, co-infection, *Culex pipiens*

Session 12: Zoonoses & Wildlife II / Abstract-ID 196

Novel paramyxoviruses are common in South African small mammals

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Paramyxoviruses are a highly abundant and diverse viral group with public and veterinary health relevance. Our study aimed to identify and characterise paramyxoviruses in putative small mammal reservoirs in South Africa, including insectivorous bats, rodents, shrews and sengis.

Species of the widely distributed and populous rodent genus *Rhabdomys*, endemic to southern Africa, were found to harbour diverse and abundant (overall prevalence 19.4% [106/546]) paramyxoviruses. L gene sequencing showed substantial viral diversity at host individual, population and species levels.

A total of 23/359 (6.4%) insectivorous bats belonging to 16 species in five families were PCR-positive, including eight species previously not implicated as paramyxovirus hosts. Cape horseshoe bats (*Rhinolophus capensis*) harbour different virus variants / strains; multiple nonsynonymous mutations resulting in amino acid changes may point to their zoonotic potential.

Four common shrew species harboured putative henipaviruses; *Crocidura flavescens* showed the sustained presence of a novel virus revealed by near full-length genome sequencing as phylogenetically closely related to the rat-borne henipavirus, Mòjiāng virus.

The abundance and diversity of novel putative paramyxoviruses discovered by this study and the implication of numerous previously unimplicated species as potential reservoir hosts reaffirms the importance and need for ongoing surveillance efforts of especially small mammals in South Africa.

Keywords:

Paramyxoviruses; henipavirus; insectivorous bats; shrews; sengis; rodents; *Rhabdomys*;

Session 12: Zoonoses & Wildlife II / Abstract-ID 264

Host-species barriers affecting LASV infection in *M. natalensis*

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Lassa virus (LASV) is a hemorrhagic fever-causing Arenavirus endemic in Western Africa. Up to 5000 deaths per year are attributed to infections with this rodent-borne pathogen.

The main rodent reservoir is the multimammate mouse *Mastomys natalensis* a commensal rodent found throughout Sub-Saharan Africa. However, in recent years several other rodent species have been identified as additional reservoir hosts. In this study we assessed the ability of several LASV strains originating from other rodent reservoirs to establish infection in *M. natalensis*.

Mastomys natalensis were infected with various LASV strains using different infectious doses. Animals were followed for up to eight weeks post-infection and blood, organs and urine were sampled at frequent intervals. Furthermore, naïve animals were co-housed with inoculated individuals to assess natural transmission.

We previously described long term persistence of LASV isolated from *M. natalensis* in its natural rodent host. Similarly, a LASV strain isolated from the closely related *M. erythroleucus* is also capable of establishing persistent infections in *M. natalensis* and is readily transmitted to exposed individuals. In contrast, LASV strains originating from non-Mastomys rodents only caused transient infections followed by seroconversion. Furthermore, these strains showed no or only limited transmission between infected animals and their contacts.

Keywords:

Lassa virus, *Mastomys natalensis*, Host-species barriers

Session 12: Zoonoses & Wildlife II / Abstract-ID 178

Virulence factors and phylogenetic relationships in *Staphylococcus aureus* from wild ungulates in Brandenburg, Germany

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Data regarding the occurrence of *Staphylococcus* (*S.*) *aureus* in wild living animals is rare. The aim of this study was to provide insights into the occurrence and characteristics of *S. aureus* in ungulates from Brandenburg, Germany.

Nasal swabs of wild boars, roe, fallow and red deer were collected in hunting season 2021/2022 and analyzed for *S. aureus* by selective enrichment. Species were determined using matrix assisted laser desorption ionization mass spectrometry. Whole-genome sequencing was conducted for genotyping and analysis of phylogenetic relationships.

S. aureus were recovered from approximately 7% of nasal swabs. *S. aureus* isolates were associated with several sequence types (ST). ST1 isolates clustered closely together in the phylogenetic tree. Genes encoding staphylococcal enterotoxin (SE) or SE-like (SEI) were found in 14/17 isolates. In particular, a *seh* gene was present in 12/17 isolates. Moreover, two isolates harbored a multiplicity of SE or SEI genes. In addition, the toxic shock syndrome toxin encoding *tsst-1* gene was detected in an isolate.

Wild animals may carry potentially virulent *S. aureus*. The close phylogenetic relationship of *S. aureus* isolates indicates a possible spread between animals from the same territory. Handling of animals or their carcasses might contribute to staphylococcal infections in humans. Moreover, food poisoning due to SE producing strains may occur, if recommended hygiene practices are not applied during processing of game meat.

Keywords:

Staphylococcus aureus, Wild Ungulates, Virulence, Toxins

Session 12: Zoonoses & Wildlife II / Abstract-ID 193

Hepatitis E virus in wild boars in Brandenburg, Germany, 2018 - 2022

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Infection with the hepatitis E virus (HEV) can cause liver inflammation in humans. The zoonotic HEV genotype 3 is endemic in Germany and can be transmitted by ingesting insufficiently cooked wild boar meat. To evaluate the risk associated with consumption of game meat, wild boar liver samples from 28 hunting areas in Brandenburg were collected between 2018 and 2022 within the framework of the BfR-Center for Land Use-Related Evaluation Methods and One Health Approaches. Samples were screened by RT-qPCR for the presence of HEV and detected strains were analyzed by whole genome sequencing. Overall, 14 of 275 samples tested HEV-positive, resulting in a mean detection rate of 5 %. However, detection rates and available sample numbers varied greatly between the hunting seasons, with 2 % (1/44) in 2018, 7 % (5/74) in 2019, 21 % (4/19) in 2020, 2 % (2/107) in 2021, and 7 % (2/31) in 2022. So far, three whole genome sequences were obtained, showing a high subtype diversity within the small investigated area of Brandenburg. Two genomes were assigned to subtypes 3e and 3h, while the third sequence showed highest similarity with a so far unclassified genotype 3 subtype. This study and continued surveillance of HEV in wild boars is important to gain an overarching understanding of the role of wild boar as animal reservoir and source for human HEV infections.

Keywords:

Hepatitis E virus, wild boar, zoonoses

Session 12: Zoonoses & Wildlife II / Abstract-ID 205

Comparison of human- and rat-derived strains of rat hepatitis E virus in cell lines of both hosts

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Rat hepatitis E virus (ratHEV) is distantly related to the well-studied human-pathogenic HEV genotypes 1-4 and distributed in rats worldwide. Recently, ratHEV infection has been demonstrated in several hepatitis cases in human patients from Hongkong, Canada and Spain. To characterize rat- and human-derived ratHEV strains in more detail, we generated genomic plasmids, which were in-vitro transcribed, capped and transfected into human hepatoma cell lines to generate infectious virus. One ratHEV strain was derived from a hepatitis patient from Hongkong, the other from a rat in Germany, and a human genotype 3 strain served as a control. Virus replication was evident for all viruses in all transfected cell lines. However, it was most robust in Huh7-Lunet BLR cells, which could also be passaged resulting in persistently infected and virus-producing cell lines. The generated viruses were used to infect human-derived PLC/PRF/5, Huh7 and Huh7-Lunet BLR hepatoma cell lines as well as rat-derived hepatoma cell lines MH1C1, clone 9 and H4IIE. All of the viruses replicated in the human cell lines, but showed no stable infection of the rat-derived cell lines. The ratHEV strain from the human patient showed a more efficient replication in the human cells as compared to the rat-derived strain, which may indicate a better adaptation to humans. Generally, the results show robust replication of ratHEV in human cell lines, which confirm its zoonotic potential for transmission to humans.

Keywords:

cell culture; ratHEV; zoonotic potential; reversed genetics system; HEV

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