Junior Scientist Zoonoses Meeting 2022

Program and Abstracts

Federal Ministry of Education and Research

German Research Platform for Zoonoses

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Welcome Note

Dear Junior Scientists of the German Research Platform for Zoonoses,

We are happy that after two years in the virtual space the Junior Scientist Zoonoses Meeting (JSZM) finally returns to the real world and we can welcome you in Hanover.
Since its first edition in 2013, the JSZM has become a central anchor of the Zoonoses Platform's work for young researchers. The meeting is an ideal networking opportunity for young scientists and offers a view beyond one's own research field on various levels. Especially in a research area as diverse as zoonoses research, where many disciplines overlap, collegial networks are valuable building blocks for future careers. In addition, the interdisciplinary exchange of knowledge is indispensable in order to be able to place one's own research in a larger One Health context.
The corona pandemic has brought a lot of movement into zoonoses research and the interdependencies of the health of humans, animals and their environment in the sense of the One Health concept have found their way into many current initiatives. As zoonoses researcher, you are already familiar with the broad outlines of the One Health concept. However, interdisciplinary research also places very special demands on young scientists, which cannot usually be addressed by university education alone.
It is therefore an important goal of the Zoonoses Platform to help young researchers from an early career stage onwards. To realize this, we offer various workshops, events, and funding formats that are specifically tailored to the needs of young researchers. With the One Health Certificate, we also offer the opportunity to receive credit for interdisciplinary engagement. We strongly believe that the interdisciplinary education of young scientists will strengthen One Health research and thus form the foundation for the successful prevention and control of zoonotic infectious diseases in the long term.
In this sense, we are very pleased that you are contributing to networked zoonoses research with your participation in the JSZM and that you are bringing the One Health concept to life as an active member of the community. The JSZM will give you the opportunity to present your own research and to receive valuable feedback, as well as to interact with established researchers in the field.
We look forward to a successful meeting and are grateful for your active contribution to the zoonoses research community in Germany.

The German Research Platform for Zoonoses

General Information

Date & Venue
June 23rd -24th, 2022
Leonardo Hotel Hannover, Tiergartenstraße 117, 30559 Hannover, Germany

Conference languages
The official conference language is English.

Organization
German Research Platform for Zoonoses
c/o Greifswald - Insel Riems, Dr. Dana Thal
zoonosenplattform@fli.de, www.zoonosen.net, info@zoonosen.net
Twitter @GZoonoses

Image rights and publications
The German Research Platform for Zoonoses reserves the right to publish images from the meeting as well as the content of the lectures on its website and in the context of follow-up reports.

Attending guest speakers

- Dr. Björn Corleis (Institute of Immunology, Friedrich-Loeffler-Institute)
- PD Dr. Nicole de Buhr (Institute of Biochemistry, University of Veterinary Medicine Hanover)
- Bob Fregin (ZIK HIKE, Centre for Innovation Competence - Humoral Immune Reactions in Cardiovascular Diseases, University Greifswald)
- Dr. Kim Grützmacher (GIZ and Museum für Naturkunde Berlin)
- PD Dr. Ellen Krautkrämer (Nierenzentrum Heidelberg, Universitätsklinik Heidelberg)
- Prof. Dr. Thomas Pietschmann (Institute of Experimental Virology, TWINCORE)
- Dr. Hendrik Scheinemann (Bundeswehr Research Institute for Protective Technologies and CBRN Protection)
- Dr. Ilia Semmler (Institute of Virology, Charité Berlin)
- Dr. Christian Sieben (Helmholtz-Centre for Infection Research)
- Prof. Dr. Lidwien Smit (Department Population Health Sciences, Institute for Risk Assessment Sciences (IRAS), Utrecht University)

Continuous Veterinary Education

The Junior Scientist Zoonoses Meeting 2022 is registered for "ATF-Stunden" by the Federal Chamber of Veterinarians (Bundestierärztekammer). You can receive your certificate at the end of the meeting.

Poster presentations
Each participant has the opportunity to present his/her poster during the meeting. In order to learn about as many posters as possible during the meeting you will be grouped into three different groups during the poster sessions. Please take a look at the Poster Session Schedule (page 6 -7) to learn about your groups. You can also find a plan of the poster exhibition area in this booklet (page 8). Additionally, the abstracts of the posters are published within this abstract volume of the meeting.

Election of the junior scientist's representative

The Internal Advisory Board of the German Research Platform for Zoonoses is the decision-making organ of the platform. It decides on project and event proposals, advises on the strategic orientation of the platform and monitors the scientific quality of the platform.

Each year the Internal Advisory Board is elected by the members of the Zoonoses Plattform. One seat in the internal advisory board is hold by an early career scientist to represent the interests of young scientists within the platform. All doctoral students as well as early career postdocs that are members of the platform can run for election.
As an elected member of the Internal Advisory Board 3 to 5 meetings have to be attended per year. Furthermore, the elected person serves as a contact person for all junior scientists within the Zoonoses Platform. The position offers the opportunity to get an impression of committee work in an established scientific organization in Germany and to promote the interest of young scientists within the community.
The election will take place during the first day of the meeting.

Joint Dinner
All participants of the meeting, who registered in advance, are welcome to join the dinner on June 22nd, as well as on June 23rd. Please note that each participant must pay for their own drinks and food at the dinner.

Funding
The Junior Scientist Zoonoses Meeting is funded by the German Federal Ministry of Education and Research.

PROGRAM JSZM 2022

OPTIONAL – JUNE 22, 2022

19:00	*Optional joint dinner*

DAY 1 – JUNE 23, 2022

FROM 8:30	Registration
9:15 – 09:30	Welcome note
09:30 – 11:00	**Career talks**
9:30	*Becoming a group leader in virology* - Thomas Pietschmann (TWINCORE)
10:00	*One Health: Science, politics and implementation* – Kim Grützmacher (GIZ and Museum für Naturkunde/ Leibniz Institute for Evolution and Biodiversity Science)
10:30	*Science management* – Ilia Semmler (Charité- University Medicine Berlin)
11:00 -11:20	Coffee break
11:20 – 12:20	**Poster session I** – Groups 1 to 5
12:20 – 13:10	Lunch
13:10 – 14:40	**Zoonoses research projects**
13:10	*Influenza-A induced bacterial co-infections (IVIBak)* – Nicole de Buhr (University of Veterinary Medicine Hannover)
13:40	*N protein based in-vitro model to characterize the risk of orthohantaviruses* - Ellen Krautkrämer (Nierenzentrum Heidelberg)
14:10	*One Health Vaccine* – Björn Corleis (Friedrich-Loeffler-Institute)
14:40 – 15:10	**Election young scientist representative**
15:10 – 15:30	Coffee break
16:00 – 17:30	**Guided tour TiHo (University of Veterinary Medicine Hannover)**
19:00	Dinner (optional)

DAY 2 – JUNE 24, 2022

9:30 – 10:30	**Keynote**
	One Health and Environmental Epidemiology – Lidwien Smit (Utrecht University)
10:30 – 11:00	Coffee break
11:00 – 11:45	**Poster session II** – Groups A to F
11:45 – 12:30	**Poster session III** – Groups α to θ
12:30 – 13:30	Lunch

13:30 – 14:30	**Methods in infection research**
13:30	*Linking the mechanical properties of cells and biological function* - Bob Fregin (University Greifswald)
14:00	*Microscopy for virus-cell-interaction* - Christian Sieben (Helmholtz-Center for Infection Research)
14:30 – 15:00	**Current affairs** *Research against weapons of mass destruction* - Hendrik Scheinemann (Bundeswehr Research Institute for Protective Technologies and CBRN Protection)
15:00 – 15:20	Coffee Break
15:20 – 16:00	Discussion + farewell

Poster Sessions – Schedule

Poster Session I – June 23, 11:20 – 12:20

Time	Name	ID	Name	ID	Name	ID
	Group 1		**Group 2**		**Group 3**	
11:20	Fischer	E3	Yasobant	R4	Boten	V3
11:40	Jahan	E5	Meyland	P5	Ernst	E2
11:00	Rauhöft	V5	Clever	I1	Abdel Aleem AL-Hosary	V1
11:10	Körsten	V4	Palacios Pedrero	I4	Finkensieper	I2
11:50	Scheinemann	R2	Pohl	N3	Goretzko	P2
11.30	Steiner	R3	Pinecki Socias	N2	Duchow	P1
	Group 4		**Group 5**			
11:20	Böhm	R1	Jaffe	E4		
11.30	Lassnig	P4	Bergmann	E1		
11:40	Hrabal	I3	Wehmeyer	V4		
11:50	Kronfeld	N1	Schreiber	P6		
11:00	Ajamma	V2	Nobel	R5		
11:10	Reemtsma	V6	Halwe	P3		

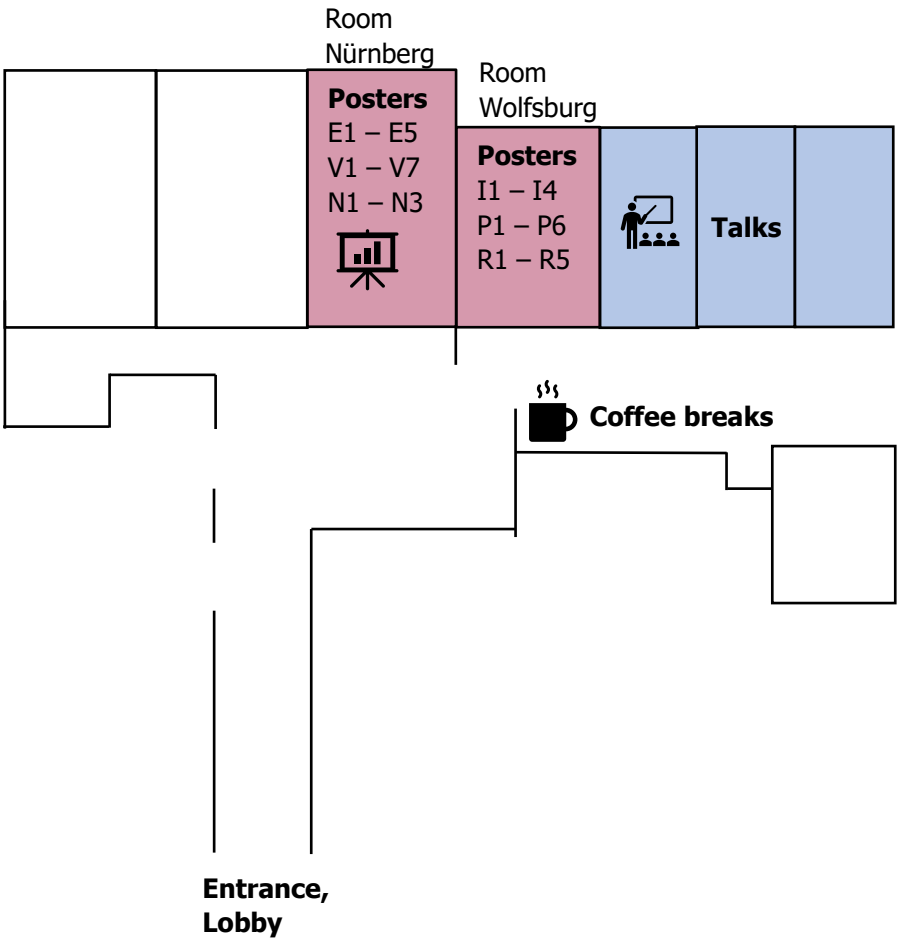
Poster Session II – June 24, 11:00 – 11:45

Time	Name	ID	Name	ID	Name	ID
	Group A		**Group B**		**Group C**	
11:00	Boten	V3	Böhm	R1	Bergmann	E1
11:09	Kronfeld	N1	Scheinemann	R2	Jahan	E5
11:18	Rauhöft	V5	Pohl	N3	Palacios Pedrero	I4
11:27	Pinecki Socias	N2	Abdel Aleem AL-Hosary	V1	Duchow	P1

11:36	Schreiber	P6	Jaffe	E4	Hrabal	I3
	Group D		**Group E**		**Group F**	
11:00	Steiner	R3	Halwe	P3	Yasobant	R4
11:09	Clever	I1	Meyland	P5	Ernst	E2
11:18	Goretzko	P2	Finkensieper	I2	Ajamma	V2
11:27	Lassnig	P4	Fischer	E3	Wehmeyer	V7
11:36	Nobel	R5	Reemtsma	V6	Körsten	V4

Poster Session III – June 24, 11:45 – 12:30

Time	Name	ID	Name	ID	Name	ID
	Group α		**Group β**		**Group γ**	
11:45	Fischer	E3	Steiner	R3	Jahan	E5
11:54	Bergmann	E1	Meyland	P5	Ajamma	V2
12:03	Boten	V3	Kronfeld	N1	Finkensieper	I2
12:12	Böhm	R1	Jaffe	E4	Clever	I1
12:21	Yasobant	R4	Ernst	E2	Schreiber	P6
	Group δ		**Group ε**		**Group θ**	
11:45	Scheine-mann	R2	Rauhöft	V5	Hrabal	I3
11:54	Palacios Pedrero	I4	Pohl	N3	Nobel	R5
12:03	Goretzko	P2	Duchow	P1	Körsten	V4
12:12	Reemtsma	V6	Lassnig	P4	Pinecki Socias	N2
12:21	Wehmeyer	V7	Halwe	P3	Abdel Aleem AL-Hosary	V1


Room
Nürnberg
Posters
E1 – E5
V1 – V7
N1 – N3
Room
Wolfsburg
Posters
I1 – I4
P1 – P6
R1 – R5
Talks
Coffee breaks
Entrance,
Lobby

Abstracts

Junior Scientist Zoonoses Meeting 2022

Overview Abstracts

Name	Title	ID	Page
Vector-borne Diseases *(room Nürnberg)*			
Abdel Aleem AL-Hosary	*Vector Competence of Aedes albopictus mosquitoes from Germany for Rift Valley Fever virus*	**V1**	**14**
Ajamma	*Impacts of rearing conditions on larval stages of important West Nile virus vectors: Culex pipiens biotypes*	**V2**	**15**
Boten	*Identification of novel West Nile virus vector factors in insect cells*	**V3**	**16**
Körsten	*Co-infections of West Nile virus and Usutu virus in Culex pipiens biotype molestus*	**V4**	**17**
Rauhöft	*Near real-time decision tool to response against emerging mosquito-borne disease*	**V5**	**18**
Reemtsma	*Pathogenesis of West Nile virus lineage 2 in domestic geese after experimental infection*	**V6**	**19**
Wehmeyer	*A meta-analysis of mosquito host-feeding patterns*	**V7**	**20**
Epidemiology and Ecology of Zoonotic Diseases *(room Nürnberg)*			
Bergmann	*Seroepidemiological survey of a long-eared owl (Asio otus) in a zoological garden in Northern Germany*	**E1**	**22**
Ernst	*Prion Protein Gene Diversity in European Cervids*	**E2**	**23**
Fischer	*Anthropozoonotic pathogens in free ranging synanthropic Egyptian geese Alopochen aegyptiaca (Linneaus 1766) in Germany*	**E3**	**24**
Jaffe	*Oesophagostomum stephanostomum causing parasitic granulomas in wild chimpanzees (Pan troglodytes verus) of Tai National Park, Côte d'Ivoire*	**E4**	**25**
Jahan	*Investigating host & pathogen diversity in large ecosystems using flies*	**E5**	**26**
Risk Assessment and Prevention *(room Wolfsburg)*			
Böhm	*Environmental and behavioural factors and their role for the spread and prevention of vector-borne diseases*	**R1**	**28**
Scheine-mann	*Researching for Chemical, Biological, Radiological and Nuclear (CBRN) Defence*	**R2**	**29**
Steiner	*Characterization of peri-domestic human - small mammal interfaces and associated disease risks*	**R3**	**30**
Yasobant	*Is intersectoral collaboration sufficient or essential in One Health in preventing and controlling zoonoses? An operational reflection from India*	**R4**	**31**

Nobel	*Exploring the (bush)meat market: gaining insight into animal & public health in Dzanga-Sangha Protected Areas, Central African Republic*	**R5**	**32**
Immunology and Vaccines *(room Wolfsburg)*			
Clever	*Correlates of Protection after SARS-CoV-2 infection in a mouse model*	**I1**	**34**
Finken-sieper	*Irradiation of zoonotic parasites with low energy electrons for the development of vaccine candidates*	**I2**	**35**
Hrabal	*Vaccine to Inhibit Autochthonous Transmission of Hepatitis E (VaccInATE)*	**I3**	**36**
Palacios Pedrero	*Aging and antiviral immune response: anti PD-1 treatment improves T cell function in older adults*	**I4**	**37**
Pathogen-Cell Interactions *(room Wolfsburg)*			
Duchow	*The effect of the intra-host specific selection of Eastern Equine Encephalitis Virus variants on fitness in vertebrate systems*	**P1**	**39**
Goretzko	*Endolysosomal cholesterol imbalance is a promising antiviral target*	**P2**	**40**
Halwe	*In vivo competitive infection and transmission of SARS-CoV-2 VoCs Alpha, Delta and Omicron*	**P3**	**41**
Lassnig	*Influenza A virus and bacterial co-infection in pigs: What is the role of neutrophil extracellular traps?*	**P4**	**42**
Meyland	*Adherence of Streptococcus equi ssp. zooepidemicus to primary cardiac endothelial cells under flow*	**P5**	**43**
Schreiber	*Infection with pathogenic hantaviruses changes the kidney proteome profile*	**P6**	**44**
NGS, Genetics, Novel Methods *(room Nürnberg)*			
Kronfeld	*Phenotypic and Genotypic Characterisation of C. perfringens isolates from Dairy Cows with a Pathological Puerperium*	**N1**	**46**
Pinecki Socias	*CRISPR-mediated genome editing as a novel method for functional genome analysis of Rhipicephalus microplus ticks*	**N2**	**47**
Pohl	*Acinetobacter spp. as a source of CRESS DNA in food of animal origin*	**N3**	**48**

Vector-borne Diseases V1 – V7

V1

Vector Competence of Aedes albopictus mosquitoes from Germany for Rift Valley Fever virus

Amira AL-Hosary[1]*, Christin Körsten[1], Mandy Schäfer[1], Birke Tews[1], Martina Abs[1], Ulrike Neumann[1], Oliver Tauchmann[1], Franziska Stoek[2], Martin Eiden[2], Cornelia Silaghi[1]

[1]Institute for Infectology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Insel Riems, Germany; [2] Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Insel Riems, Germany ; *Presenting author

Keywords: RVFV-MP12, Aedes albopictus, Germany

Rift Valley fever virus (RVFV; family Phenuiviridae) is transmitted by different mosquito species and causes severe disease in both humans and livestock in many African countries. Some European countries are also at risk of its emergence because of passive movement of infected vectors. Asian tiger mosquitoes (Aedes albopictus) are globally expanding which concerns public health authorities. They were discovered recently in southwest Germany with confirmed overwintering. We investigated the vector competence of German Aedes albopictus (AA) mosquitoes for RVFV Strain MP12 under laboratory conditions at two different temperatures. One at 28°C using 313 females and second one at 32°C using 318 females. All mosquitoes were offered heparinized bovine blood mixed with 107 TCID50/ml RVFV strain MP-12 using cotton sticks. Blood-fed females were sorted and incubated for 14 days post infection (DPI). The feeding rates were 34.5% and 39.9%, the survival rates were 46.0% and 13.4%, respectively. Salivation and dissection of wings/legs were performed on 17 females from each group, followed by RT-qPCR to confirm the presence of RVFV RNA in the examined samples. The infection rates (virus RNA positive bodies) were 17.7% and 23.5%, dissemination rates (positive wings/legs) were 5.9% and 23.5%, and transmission rates (positive saliva) were 11.8% and 23.5%. This finding indicates that German AA are competent vectors for RVFV. Rising global temperatures may play a role in the further establishment of this species in Germany and may increase the risk for introduction and establishment of RVFV in Germany.

V2

Impacts of rearing conditions on larval stages of important West Nile virus vectors: *Culex pipiens* biotypes

Yvonne Ajamma[1]*, Ana Vasić[1,2], Marlene Hausner[1], Oliver Tauchmann[1], Mandy Schäfer[1], Birke Andrea Tews[1], Cornelia Silaghi[1]

[1]Institute of Infectology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany; [2]Current affiliation: Institute for Medical Research, National Research Institute of Serbia, University of Belgrade, Serbia; *presenting author

Keywords: Rearing containers; Vector competence; mosquito populations; Overcrowding; Larval feed

Culex pipiens mosquitoes are implicated as important vectors of West Nile virus (WNV), which might be partially due to their abundance and geographic distribution. The WNV can cause illness and deaths in humans and animals in affected countries. Furthermore, different WNV isolates originate from different countries, including the sub-Saharan Africa. Larval competition and overcrowding affect adult density and longevity in other mosquito species. However, there is little information on the effect of larval rearing in extreme conditions on the vector competence of *Cx. pipiens* biotypes. Therefore, this study aims to determine the effect of larval overcrowding and varying feed quantities on the vector competence of *Cx. pipiens* for WNV lineages 1 and 2. In triplicates, 10 larvae each from laboratory-reared *Cx. pipiens molestus* and two hybrids from different locations were fed with 11 different quantities (0.00001g/larva – 0.006g/larva) of Tetramin fishmeal and observed for pupal density. Overfeeding was reported as 0.003g/larva, normal feeding was 0.001g/larva, and starvation was 0.0005g/larva. These feed quantities affected the abundance of the resultant adults. Furthermore, the normal feed quantity was fed other larvae in 23-different-sized containers (25ml – 24L) to simulate possible overcrowding, and observed for survival. The container sizes had no significant impact. In a next step, resultant adults from different upbringing conditions will be artificially fed with WNV-infected animal blood and their vector competence will be assessed. In conclusion, this is an on-going baseline study to enhance the knowledge on environmental factors that influence the vector competence of WNV strains for future mosquito control planning.

V3

Identification of novel West Nile virus vector factors in insect cells

Lea Boten*[1], Birke Andrea Tews[1]

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Keywords: West Nile virus; CRISPR-Cas9 knockout, Vector factors

West Nile virus (WNV) is a small, enveloped, positive-stranded RNA virus, which is transmitted in a bird-mosquito-bird life cycle with humans and other mammals as dead-end hosts. West Nile virus is the causative agent for West Nile fever and in rare cases encephalitis. It is a member of the Flaviviridae family and as such it is closely related to other arthropod-borne viruses like Japanese encephalitis virus or yellow fever virus. Together these mosquito-borne diseases pose a major global health threat and are considered to be emerging or re-emerging diseases. WNV was initially detected in 2018 in birds in Germany and in mosquitoes in 2019. Since then a growing number of cases in horses, birds and humans have been detected. Research focused on host factors involved in WNV infection has been predominantly done in mammalian cells, data concerning factors involved in WNV infection in insect cells are scarce. To fill this gap of knowledge, our project aims to adapt the genome editing method CRISPR-Cas9, to generate directed knockouts in hard to transfect insect cells, with the overall aim to identify novel WNV host factors within these cells.

V4

Co-infections of West Nile virus and Usutu virus in *Culex pipiens* biotype *molestus*

C. Körsten[1]*, A.A. Al-Hosary[1], C.M. Holicki[2], M. Schäfer[1], B.A. Tews[1], A. Vasic[1,3], U. Ziegler[2], M.H. Groschup[2], C. Silaghi[1]

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Keywords: West Nile virus, Usutu virus, Co-infections, Culex pipiens biotype molestus

The zoonotic flaviviruses West Nile virus (WNV) and Usutu virus (USUV) have been circulating in Germany for several years, causing outbreaks in both humans and animals. Mosquitoes play a central role as biological vectors in the transmission of WNV and USUV. Due to the increasing overlap of their distribution areas, co-infections of both viruses are to be expected. However, it is largely unknown what effect co-infections could have on the vector competence of German mosquito species. To determine this impact, mosquitoes of a laboratory colony of *Culex pipiens* biotype *molestus* were orally infected with WNV lineage 2 and USUV Africa 3 or Europe 3 in mono- and co-infections. *Culex pipiens* biotype *molestus* were vector-competent for both USUV and WNV in mono-infections. Transmission efficiency (virus-containing saliva / surviving mosquitoes) of WNV appeared unaffected by a simultaneous presence of USUV. However, mosquitoes showed a reduced transmission efficiency for USUV (from 5/8 to 0/9) when co-infected with WNV and USUV Europe 3. In contrast, in mosquitoes co-infected with WNV and USUV Africa 3, no significant difference in transmission efficiency of USUV in mono- and co-infections was observed (1/7 and 3/8, respectively). Two co-infected mosquitoes had RNA of both viruses in their saliva, making co-transmissions of both viruses conceivable. Our results indicate that USUV transmission might be decreased by a simultaneous infection with WNV. However, it is possible that the observed differences can be attributed to the small number of mosquitoes examined to date. In order to validate these results, further experiments are necessary.

V5

Near real-time decision tool to response against emerging mosquito-borne disease

Leif Rauhöft[1]*, Felix G. Sauer[1], Jonas Schmidt-Chanasit[1,2] , Renke Lühken[1]

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Keywords: Vector, Ecology, Mechanistic Model, Virus transmission

Mosquitoes are well known for their ability to transmit pathogens. These include a variety of arthropod-borne viruses (arboviruses) of medical and veterinary interest. In cause of 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. In Germany, Usutu virus and West Nile virus are the two most important mosquito-borne arboviruses, predominantly transmitted by *Culex pipiens s.l./torrentium*, which are common in Germany and mainly found in and around human settlements. The aim of this project is to create a near real-time decision tool to response against mosquito-borne pathogens. From 2021 to 2023, a nationwide study will be conducted to assess the population dynamics of *Culex* mosquitoes at more than 30 different sampling sites in Germany. Therefore, mosquitoes are collected with a novel trapping system (BG-Trap Station incl. BG-Counter 2, Biogents, Regensburg), providing real-time mosquito and weather data. In addition, a mechanistic model will be developed to predict the spatiotemporal patterns of vectors and viruses on a national scale and deliver timely and local risk assessments, which will be made publicly available. By comparing the model output with the nationwide mosquito collection data, the model accuracy can be determined and further refined.

V6

Pathogenesis of West Nile virus lineage 2 in domestic geese after experimental infection

Hannah Reemtsma[*†], Cora M. Holicki[†], Christine Fast, Felicitas Bergmann, Martin Eiden, Martin H. Groschup, and Ute Ziegler

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany; * Presenting author; † Contributed equally

Keywords: West Nile virus, lineage 2, geese, pathogenesis, experimental infection

West Nile virus (WNV) is an emerging infectious zoonotic pathogen circulating between mosquitoes and birds with mammals as dead-end hosts. Since its first detection in Germany in 2018, WNV has become autochthonous, causing high mortality rates in avian species and occasional diseases in humans and horses.

To determine the possible role of free-ranging geese as amplifying hosts, 15 three-week-old domestic geese were infected subcutaneously with WNV lineage 2, an isolate from Germany from 2018 (acc. no. MH924836). The geese were sampled regularly, euthanized at various time points up to 21 days post infection (dpi), and a gross examination was performed. Subsequently, a detailed virological and histopathological / immunohistochemical examination was done. By real-time quantitative polymerase chain reaction and virus titration, virus was detected in all of the examined organs at early time points. Also, by immunohistochemistry, viral antigen was found in the brain and in the enteric nervous system of several geese as well as nonsuppurative encephalitis and ganglioneuritis.

This study provides interesting information on the organ distribution and pathohistological lesions of WNV during the course of infection in geese. The more detailed immunopathogenesis of WNV (e.g. in the brain) requires further research.

V7

A meta-analysis of mosquito host-feeding patterns

Magdalena Laura Wehmeyer[1]*, María José Tolsá García[2], Jonas Schmidt-Chanasit[1,3], David Roiz[4], Renke Lühken[1]

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Keywords: Host-feeding patterns, mosquitoes, meta-analysis

Mosquito host-feeding patterns are an important factor shaping the mosquito's vector capacity. As they determine transmission cycles and risk of pathogen spill over, the understanding of host selection is important in risk assessment for human and animal health. Host selection can depend on intrinsic (e.g. mosquito genetics) as well as extrinsic factors (e.g. host availability). For example, anthropophilic mosquitoes are potential vectors for pathogens transmitted between humans (e.g. chikungunya virus), while opportunistic mosquitoes can serve as bridge vectors for zoonotic viruses (e.g. West Nile virus). In order to investigate mosquito host-feeding patterns, we collected the data from 295 scientific studies, covering a timeframe of nearly seven decades (1942-2019). We included studies which sampled engorged mosquito females and screened the bloodmeal for hosts using any serological or molecular biological method. The collected and standardized parameters comprised mosquito species, blood meal hosts, collection method, method for blood meal analysis, time and date, and, if provided, land use and landscape information per study. These data on 545.364 mosquito specimens allow wide range of further analysis. For example, 277 of the mosquito taxa (57.67%) fed on humans, making them potential vectors of pathogens relevant for public health. Furthermore, the data indicate different host-feeding patterns: while some mosquito species like Culex quinquefasciatus show a broad host range, clear preferences for non-human mammalian species are evident for Culex tritaeniorhynchus. This comprehensive meta-analysis will help to understand transmission risk of mosquitoborne pathogens and to conduct vector-specific control measures in the future.

Epidemiology and Ecology of Zoonotic Diseases E1 – E5

E1

Seroepidemiological survey of a long-eared owl (*Asio otus*) in a zoological garden in Northern Germany

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Keywords: Usutu virus, zoo birds, Germany, monitoring

Zoo birds play an important role as sentinels. They are location-based, in close contact to urban areas, monitored daily, and often very susceptible to viruses. Therefore, we conduct monitoring including follow-up studies of zoo birds to assess the emergence and spread of flaviviruses like West Nile virus (WNV) and Usutu virus (USUV).

For the last ten years, USUV has been circulating in Germany. In 2018, there was a massive spread of the virus throughout Germany combined with a high fatality rate in wild and captive birds. By contrast, WNV reached Germany only recently.

A follow-up study took place in "Wildpark Schwarze Berge". Blood of zoo birds was drawn before the mosquito season in spring and after the season in autumn over a period of three years. Extracted RNAs were analyzed by RT-qPCRs specific for USUV and WNV genomes. The serum samples were screened by a commercial ELISA as well as by virus neutralization assays.

A long-eared owl kept in "Wildpark Schwarze Berge" was especially interesting as we were able to show the course of specific antibodies against USUV over the entire sampling period (excluding autumn 2020). In spring 2021, we isolated specific USUV genomes from the long-eared owl's blood coagulum. A whole genome sequence was generated using MinION Nanopore sequencing and USUV-specific amplicons.

Our aim is to follow the progression of antibody levels in naturally USUV-infected birds to enable an insight into antibody persistence and development after reinfection.

E2

Prion Protein Gene Diversity in European Cervids

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Keywords: Chronic Wasting Disease, prion protein gene, cervids

Chronic Wasting Disease (CWD), a Transmissible Spongiform Encephalopathy caused by the misfolding of a cellular prion protein (PrP^{C}) into its pathological isoform (PrP^{CWD}), has been endemic in North American cervids since the 1960s. However, the disease has recently also emerged in Scandinavia not only in its contagious but also in a novel 'atypical' form. CWD now threatens to spread throughout the European continent. Since susceptibility to this disease is largely determined by the structure of the prion protein gene (*PRNP*) of the host, it is necessary to know the diversity of the *PRNP* to estimate the threat CWD poses to the European deer population. Therefore, samples from 3000 red deer (Cervus elaphus), roe deer (*Capreolus capreolus*) and sika deer (*Cervus nippon*) were collected throughout Germany and in smaller numbers from neighbouring states. Sequencing of the Open Reading Frame (*ORF*) of the *PRNP* will provide information on the variations in genotype frequencies and their geographical distribution. The identified genotypes will be interpreted in the context of previously known sequencing data from North American and European studies, as well as the results of experimental deer challenge studies. This study will provide new insights into the vulnerability of European cervid populations and improve European surveillance and control measures.

E3

Anthropozoonotic pathogens in free ranging synanthropic Egyptian geese *Alopochen aegyptiaca* (Linneaus 1766) in Germany

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Keywords: Echinostomiasis, Salmonella spp., Cryptosporidiosis, synanthropic waterfowl

Egyptian geese (*Alopochen aegyptiaca*) are neozootic Tandorninae in Europe. The birds prefer urban habitats like parks or public swimming pools. Contact between humans, their pets and Egyptian geese is frequent. This study presents the first large scale investigation of anthropozoonotic parasites and bacteria in these birds in Germany. Egyptian geese were searched in their possible habitats over a period of eighteen months. Scat samples were collected whenever defecation occurred (*n* =148). The samples were examined on parasitical eggs by standard sodium acetate acetic formalin-technique. 114 shot Egyptian geese were examined in necropsy. 96 scat samples were examined on *Cryptosporidia* spp. by the karbol-fuchsin-technique, with and coproantigen-ELISA and by qPCR. In total 138 collected samples were investigated on *Salmonella* spp.. 14 public pools in six different towns were sampled intensive on *Salmonella* spp.. They were examined by the standard ISO 6579 (2002) or a combination of pre-enrichment and qPCR.
All examined samples were negative on *Salmonella* spp.. Single oocysts of *Cryptosporidia* spp. were found in 4,17% of the samples in microscopy. 8,84% of all carcasses carried adult Echinostomatidae. These are cosmopolitan anthropozoonotic parasites of the small intestine. A segment of the mitochondrial *cox-1* gene and the mitochondrial *ND1* gene were amplified and sequencend to determine the species. 7,28% of the scat samples contained eggs of Echinostomatidae. Regarding *Salmonella* spp. and *Cryptosporidia* spp. the anthropozoonotic risk originating from Egyptian geese in Germany seems to be low. Still there is a clear risk of infection with Echninostomiasis.

E4

Oesophagostomum stephanostomum causing parasitic granulomas in wild chimpanzees (*Pan troglodytes verus*) of Tai National Park, Côte d'Ivoire

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Keywords: Helminths; parasites; parasitic granuloma; Oesophagostomum stephanostomum; nodular worms; primates

Oesophagostomum sp. is a parasitic nematode that frequently infects primates across widely separated field sites in Africa and Asia. In humans, nodular lesions in the abdomen caused by *O. bifurcum* are common in certain areas of Togo and Ghana. Similar granulomas have been observed in chimpanzees, gorillas and baboons post-mortem. At Tai National Park (Côte d'Ivoire), previous research in wild chimpanzees (*Pan troglodytes verus*) has uncovered a variety of *Oesophagostomum* spp. larvae in stool, and nodular lesions associated with unspecified *Oesophagostomum* sp. post-mortem. This study describes three recent cases of parasitic granulomas found post-mortem in the intestinal tract and abdominal wall of chimpanzees at Tai. Descriptions of gross pathology, histopathology and parasitology are complemented by new molecular results obtained by PCR and sequencing of DNA isolated from the parasitic nodules. Histologically, all three animals showed chronic colitis with granulomatous inflammation consisting of macrophages, neutrophils and eosinophils, with an external capsule formed by connective tissue. The larval ITS-2 region (Internal Transcribed Spacer) sequences obtained from the nodules matched (100%) *O. stephanostomum*. Samples from the three Tai chimpanzees were 100% identical to each other and 99-100% similar to *O. stephanostomum* reported from monkeys, apes and humans in Kenya, Uganda, Gabon, Cameroon, Democratic Republic of the Congo, and Tanzania.

E5

Investigating host & pathogen diversity in large ecosystems using flies

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Keywords: Ecosystem, Biomonitoring tool, Fly legs DNA, Biodiversity, Bcbva ecology

Ecosystems have been changing due to the massive biodiversity crisis, emergence and re-emergence of potential pathogens and increased disease outbreaks. Therefore, it is crucial to know what is happening inside these ecosystems. Here, we present a rapid biomonitoring tool to keep track of ecosystems. Using flies as an established biomonitoring tool we have a cost-effective biomonitoring tool for the unbiased detection of mammalian and pathogen diversity in different ecosystems. Traditional fly extraction methods are time intensive and we were interested if there might be other ways to make fly-based biomonitoring system cheaper and faster to scale-up sample throughput and reduce per sample costs. Instead of extracting DNA from whole flies we explored the possibility to use fly legs. By using one leg per fly and pooling ≤100 legs per extraction we were able to increase throughput and reduce per sample costs by factor 100. Using metabarcoding approaches, we were able to describe mammalian diversity (16S mtDNA), eukaryotic pathogen community (18S-V9 DNA) and fly species community (CO1) from 1150 fly leg pools (100 legs/pool) collected from the Kibale national park (Uganda). Using the fly-based biomonitoring system, we were also able to map the spatial and genomic distribution of forest anthrax (*Bacillus cereus* biovar *anthracis*) in Täi National Park (Côte d'Ivoire) and it's periphery. By demonstrating the use of fly legs as biomonitoring tool to describe mammalian diversity and associated pathogen communities we can contribute to understand specific disease ecology and the intensified biodiversity crisis with cost-effective and easy to implement methods.

Risk Assessment and Prevention R1 – R5

R1

Environmental and behavioural factors and their role for the spread and prevention of vector-borne diseases

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Keywords: vector-borne diseases, tick-borne infections, emerging diseases, disease prevention

Occurrence of vector-borne diseases in humans is highly dependent on both environmental conditions and human behaviour. Understanding patterns and interactions provides options for targeted prevention. The research focus is on common endemic vector-borne infectious diseases. In Germany and Bavaria, this mainly concerns Lyme borreliosis (LB) and tick-borne meningoencephalitis (TBE). Notification data provides a basis for disease monitoring; specific studies complement these data. Objectives of two studies - one survey among LB cases, one among physicians in outpatient care - are to identify knowledge gaps or misbeliefs as well as reasons or barriers for applying and promoting prevention, in order to provide information for effective and targeted education and intervention measures. Aim of another study was to estimate the *Borrelia burgdorferi*-specific seroprevalence among children and adolescents in Germany, the rates of seroconversion and seroreversion and to assess associated factors. A secondary focus is on imported vector-borne diseases, especially those transmitted by mosquitos. While the thread may seem rather theoretical currently, it is expected to play a more important role over time through effects of climatic change, global trade and passenger travel. Imported tiger mosquitoes have repeatedly established in Bavaria. Autochthonous acquired West Nile virus infections, with the native Culex mosquito playing a central role for transmission, have been recorded in Germany since 2018, including in Bavaria. The aim is to plan and formulate recommendations for prevention of further spread. The aim is to combine individualised, behaviour-oriented approaches to primary prevention of endemic diseases with prevention and control of imported vector-borne infections.

R2

Researching for Chemical, Biological, Radiological and Nuclear (CBRN) Defence

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Keywords: verification, decontamination, protection, dual use, water treatment

Since 40 years, the threat of weapons of mass destruction (WMD) was only marginally noticed in the German public perception. This is deceptive, because the number and variety of threats has grown. The CBRN threats we face have diversified, as have the spectrum of state and non-state actors with access to WMDs and the demonstrated willingness to employ them on the battlefield and in targeted assassinations. Due to Russia's invasion of Ukraine in February 2022, the use of CBRN Weapons even on European battlefields is no longer unthinkable. The WIS located in Munster (Lower Saxony) with its experts against CBRN threats, is the German point of contact for technical protection against WMDs. The research institute is in close contact with decisions makers, other federal research institutes as well as further domestic and international institutions to study, advice and support. Part of our international work is the maintenance of an Organisation for the Prohibition of Chemical Weapons (OPCW) approved reference laboratory to confirm the prohibited use of chemical weapons as happened in 2020, 2018 or 2013 together with our colleagues from Munich. An effective way to prevent the use of the most terrifying weapons of mankind is the ability to hold perpetrators accountable. Moreover, innovations offer promising new and enhanced capabilities that can support our CBRN defence capabilities, including enhanced approaches to detection, protection, decontamination and medical treatment. For those significant tasks, a mixed team of highly trained scientists like physicists, chemists, biologists, biochemists, physicians, veterinarians and engineers are needed.

R3

Characterization of peri-domestic human - small mammal interfaces and associated disease risks

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Keywords: (peri-)domestic biodiversity – indoor environmental DNA – reservoir host contact – spill over risk

This project will characterize the diversity of bats, rodents and shrews (small mammals, SM) in human houses, stables, and gardens, look for associations with building features and correlate this with different SM pathogen prevalence in one site in Taï, Cote d'Ivoire. For animal detection, we will use metabarcoding coupled with a set of highly scalable environmental DNA (eDNA) methods.
In the first phase, we will compare the SM detection rates of already published eDNA methods in our specific setting to classical detection tools. We hypothesize that the eDNA methods can at least detect as many species as classic tools.
Secondly, we assume that SM species can be detected in and around nearly every house in Taï and that presence is promoted by good building accessibility, food availability and the absence of domestic predators. Therefore, we will take eDNA samples in and around randomly selected houses including detailed documentation of each site. Using our metabarcoding tools and our site documentation we will correlate SM presence to specific features of sampled houses. Thirdly, we will test different pathogen prevalence levels in SMs in and around houses. The prevalence and the detection rate of their SMs hosts enables us to estimate peri-domestic human exposure likelihood to these pathogens, also in correlation to features of houses. Precisely, we will use samples from SMs specifically captured for this study and existing samples. Individuals of species that were detected by eDNA methods in sampled houses will be tested with generic PCR systems.

R4

Is intersectoral collaboration sufficient or essential in One Health in preventing and controlling zoonoses? An operational reflection from India

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Keywords: One Health, Zoonoses prevention, Intersectoral collaboration, Health system

One Health (OH) emphasizes collaboration, consensus, and partnership across actors and sectors from the interface of the human, the animal, and the environment for optimal health for all. So far in OH, the intersectoral collaboration (ISC) remained the key for implementation, especially in prevention and control zones. Although evidence indicates that OH implementation relies on collaboration across diverse sectors and actors; on the other hand, there is a lack of understanding of the required level of integration due to differences in health system structure, responsiveness, and accommodative culture of the actors makes zoonoses prevention challenging. A primary study entitled Research to explore Intersectoral collaboration for One Health (RICOHA) was conducted in Ahmedabad, a western city of India, during 2017-20. The health system structure (both human and animal) and its structural competency were assessed for prevention and control of zoonoses (Rabies, Brucellosis, Swine & Bird flu), and data were collected at three different levels of the health system i.e., managerial and/or policymaker level, provider and/or clinical level and community level (both from provider and recipient perspective). The mixed data were analyzed to investigate the necessity of ISC in a local operational context. This study highlights the necessity of ISC for zoonoses prevention & control through an integrated OH approach. The ISC in the form of degree of centralization and network density varied during the bird flu outbreak control and during the non-outbreak period for zoonoses prevention. There were significant differences in the interest and influence matrix of the stakeholders across three different zoonoses. The awareness level also varies, with the highest in Rabies control and lowest in Brucellosis prevention among community health care workers and community members.

R5

Exploring the (bush)meat market: gaining insight into animal & public health in Dzanga-Sangha Protected Areas, Central African Republic

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Keywords: zoonotic disease, bushmeat, Central African Republic, wildlife, livestock

A better understanding of (zoonotic) pathogens and potential sources of infection present in the area of Dzanga Sangha Protected Areas (DSPA) can help to protect the health of human and livestock as well as that of endangered wildlife in future. The project is therefore relevant for both conservation, veterinary and public health in the Central African Republic.

This project will investigate the presence of important (zoonotic) pathogens that pose a threat to both human and livestock as well for wildlife in DSPA. In order to assess the presence of (zoonotic) diseases in wildlife and livestock, we (i) investigate the bushmeat hunting practices, (ii) collect soil, flies and airborne environmental DNA samples at the (bush)meat market. Additionally, human fecal samples are collected from latrines and screened for the presence of mammal DNA of wildlife species. Lastly, (iii) we will carry out a serosurvey on important livestock diseases in goats and sheep and conduct a questionnaire on livestock production and (bush) meat consumption by villagers of Bayanga, Central African Republic.

Immunology and Vaccines I1 – I4

I1

Correlates of Protection after SARS-CoV-2 infection in a mouse model

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Keywords: SARS-CoV-2, COVID-19, Correlates of Protection, K18-hACE2 mice

In the ongoing COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many countermeasures are already established, such as therapeutic measures or vaccine development. However, the knowledge about the immunopathology of SARS-CoV-2 and the correlates of protection after infection is still limited. The latter should play a key role in the outcome of SARS-CoV-2 infection, since we detect ranges from mild to severe symptoms in humans. Thus in this project the pathogenesis and immunological components, interacting during a SARS-CoV-2 infection, will be characterized in a transgenic ACE2 mouse model (K18-hACE2). Those transgenic mice are expressing the human ACE2 receptor and thus are susceptible to a SARS-CoV-2 infection. For this, mice were infected intranasally with SARS-CoV-2 and monitored for clinical symptoms, the viral load and the immune responses. The correlates of protection are representing a major role in this investigation, focusing on the activation of SARS-CoV-2 specific immunity with humoral and cellular components. For this purpose it is important to investigate SARS-CoV-2-specific T cells and neutralizing antibodies. Additionally, the formation of neutrophil extracellular traps (NETs) by neutrophils will be analysed after infection. Those immune components are underlining the relevance of the combined characterization of innate and adaptive immunity for this project. The evaluation of the impact of these selected immune responses in immunopathology and immunoprotection will also contribute to further parts of this project focusing on the development and enhancement of innovative vaccines using the MVA-based vaccination strategy.

I2

Irradiation of zoonotic parasites with low energy electrons for the development of vaccine candidates

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Keywords: Toxoplasma gondii, Cryptosporidium parvum, Attenuated Vaccines, Irradiation

Cryptosporidium parvum and *Toxoplasma gondii* are two important zoonotic apicomplexan parasites. In most cases, infection with either pathogen is asymptomatic but can be dangerous for immunocompromised people. *C. parvum* is a major cause for diarrhoea-associated deaths and infection with *T. gondii* during pregnancy can cause harm to unborn children. Vaccination could help to protect people from infection with these parasites and, in veterinary use, cut off transmission routes. Currently there are no vaccines available against them, with a high demand for innovative approaches in vaccine development. A challenge developing vaccines against parasites is their complex life cycle in which they undergo severe changes in their antigen composition. For an efficient protection, vaccines have to induce immune responses against a variety of these antigens. By attenuating them, the pathogens can keep enough metabolic capacity and virulence to undergo changes in their antigen composition without causing disease symptoms.

One approach for pathogen attenuation is treatment with ionizing radiation. Nucleic acids are mainly targeted and damaged, so that protein structures stay intact. We focus on the development and optimization of a process using low-energy electron irradiation (LEEI) for the treatment of pathogens in liquid solution. By adjusting the radiation dose, the parasites stay active enough to cause a subclinical infection and induce protective immune responses. We have developed processes to treat *T. gondii* and *C. parvum* with LEEI and are currently investigating the attenuated parasites *in vitro*, analysing intracellular replication, antigen conservation and their capacity to change into different life stages after LEEI-treatment.

I3

Vaccine to Inhibit Autochthonous Transmission of Hepatitis E (VaccInATE)

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Keywords: HEV3, Hepatitis, OneHealth, Vaccine, Hepatitis E

Being a widespread pathogen, Hepatitis E virus (HEV) is the most common cause of hepatitis worldwide. While in Asia and Africa genotype 1 and 2 viruses are endemic, infections in Germany are mainly linked to genotype 3 viruses. Pigs and wild boar are known to be the main reservoir of HEV3 with a prevalence up to 98% in life stocks. Infections are transmitted zoonotically and mostly attributed to consumption of contaminated meat products or close contact to infected animals, leading to estimated 417000 human seroconversions per year in Germany. Usually HEV3 is asymptomatic or causes mild and subclinical courses within healthy patients. However, affecting immunosuppressed individuals HEV3 can trigger a severe acute or chronic hepatitis with liver fibrosis, cirrhosis as well as extrahepatic manifestations, which can all lead to life threatening conditions. 15% of these severe diseases are resistant to available therapeutics. Therefore, the VaccInATE project steps in with the aim to perform a proof-of-concept study to evaluate different vaccination strategies of pigs for HEV. Thus, the transmission of HEV to humans could consequently be prevented - supporting the One Health idea of this application. The second part of the project aims to determine anti-HEV IgG seroprevalence in the population of Pomerania by using blood samples of the well-controlled SHIP cohort (population-based project Study of Health in Pomerania), followed by in depth analysis of antibody profiles. In addition, underlying epidemiological data will be spotted and extrahepatic manifestations identified.

I4

Aging and antiviral immune response: anti PD-1 treatment improves T cell function in older adults

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Keywords: aging, immune response, influenza, vaccines, check-point inhibitors

Immunosenescence and exhaustion are age-associated processes affecting the immune system, which contributes to increased susceptibility of older adults to viral and bacterial infections, increased severity of disease, and to weaker immune responses upon vaccination. Alteration on immune cell numbers and markers are known to define such processes, including a general up-regulation of checkpoint molecules. The present study aimed at obtaining better understanding of the mechanisms leading to increased susceptibility to viral infections and reduced response to vaccination in older adults, which may aid the development of novel strategies to improve immune function in this age group. In addition, we investigated the role of inhibitory molecules in causing enhanced susceptibility to viral infections, and evaluated the use of checkpoint inhibitors (e.g., Nivolumab) to improve T cell function in the elderly. To this end, we used PBMCs from study subjects of various ages (19 - 70 years old) and characterized the main phenotypical alterations of immune cells during aging, as well as their effector functions in response to *in vitro* stimulation with different viruses (influenza virus, RSV and "common cold" coronaviruses). The main changes found during aging include reduced numbers of naïve T cells and $CD27^{+}CD28^{+}$ T cells, and increased numbers of T regulatory cells, senescent cells, and $PD1^{+}$ T cells. Interestingly, in the elderly, IFN-γ production in response to stimulation with influenza virus antigens inversely correlated to the frequency of T regulatory cells. Furthermore, treatment with an anti PD-1 monoclonal antibody (Nivolumab) increased cytokine production by influenza virus-specific T cells.

Pathogen-Cell Interactions P1 – P6

P1

The effect of the intra-host specific selection of Eastern Equine Encephalitis Virus variants on fitness in vertebrate systems

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Keywords: Alphavirus, Arbovirus, EEEV, Europe, Evolution

The Eastern Equine Encephalitis Virus (EEEV; family *Togaviridae*, genus *Alphavirus*) complex is a zoonotic arthropod-borne RNA virus causing severe neurological diseases resulting in long-term sequalae and death in humans (30 – 70%) and equids (> 90%) in the Americas. Primarily, EEEV is maintained in an enzootic cycle between *Culiseta melanura* mosquitoes and passerine birds. However, other mosquito species such as *Aedes albopictus* and *Ae*. *japonicus*, which are invasive species in Europe or *Ae. aegypti* were identified as bridge vectors, carrying EEEV to humans, equids and other vertebrates. Alphaviruses replicate rapidly with high mutation rates generating genetically diverse virus populations within a host. Thereby, the intra-host selection and diversification of viral variants is mosquito species dependent. To date, less is known about vector competence of abundant European mosquito species like mosquitoes of the *Culex* genus for EEEV and therewith, the intra-host specific selection of EEEV variants in these mosquitoes. This study shows, that the native European mosquito species *Cx*. *pipiens pipiens* and *Cx*. *torrentium* are no vectors for EEEV referentially to *Ae*. *aegypti*. Interestingly, vector competence of *Ae*. *aegypti* was temperature independent at 18°C±5°C, 21°C±5°C, 24°C±5°C, 27°C±5°C. To investigate the intra-host specific selection of EEEV variants in mosquitoes, the genetic diversity of viral populations in midguts and saliva are compared with populations of the whole body and the input virus strain, using Next Generation Sequencing. Viral growth kinetics of isolated variants are comparative characterised in an established cell culture-based readout system with vertebrate cell lines, conclusively picturing viral evolutionary process in mosquitoes.

P2

Endolysosomal cholesterol imbalance is a promising antiviral target

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Keywords: endolysosomes, cholesterol, enveloped viruses, antiviral

Viral infections are a major health, economic and social problem. Modern, globalized human lifestyles, characterized by environmental degradation, climate change and overpopulation, have greatly increased the risk of new viruses emerging and spreading. In addition to the development of direct antiviral drugs and vaccines, the development of host-targeted drugs is increasingly receiving attention in the development of novel therapies. An advantage of this approach is the reduced risk of viral resistance and a universal effect on a whole range of viruses. We are exploring the endocytotic entry of enveloped viruses (such as influenza, SARS-CoV-2, EBOV) into the cell. We identified endolysosomal cholesterol accumulation to be a host cell-protective mechanism that impairs the fusion of the viral and the endolysosomal membrane during virus escape to the cytosol. Endolysosomal cholesterol accumulation was provoked by e.g. treating cells with the antifungal itraconazole. Itraconazole treatment impaired Influenza virus infection in vitro and in vivo. Furthermore, it showed additive antiviral capacity against Influenza viruses when used in a combination therapy approach with the antiviral neuraminidase inhibitor oseltamivir. By using clinically approved drugs that shift cholesterol pools within the cell towards endolysosomes (e.g. itraconazole, fluoxetine), we described in vitro antiviral properties against novel SARS-CoV-2 as well as EBOV. We therefore suggest pharmacologically induced endolysosomal cholesterol imbalance to be thought of as a possible antiviral target against a variety of different enveloped viruses.

P3

In vivo competitive infection and transmission of SARS-CoV-2 VoCs Alpha, Delta and Omicron

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Keywords: SARS-CoV-2, ferret, hamster, competitive transmission, VOC fitness

Competitive infection and transmission experiments of SARS-CoV-2 variants have become an essential tool to study fitness of newly emerging VOCs. Importantly, full fitness characterization of these VOCs requires the usage of several animal models. Here, we investigated fitness (dis-)advantages between VOC Alpha, Delta and Omicron in the Syrian hamster and ferret animal model. Six donor animals in separated cages were inoculated with an equal ratio of either Delta and Alpha or Delta and Omicron, followed by introduction of a contact animal to generate a pairwise transmission setup in both species. The animals were kept until 21dpi, allowing serological analysis. Only in the hamster setup, donor animals were euthanized 4dpi, followed by introduction of a second contact hamster to the first contact animal. VOC ratio at specific timepoints was determined via variant-specific RT-qPCR of nasal wash or organ samples. We found that Alpha fully predominated over Delta in replication and transmission in hamsters, while we observed complete Delta predominance in ferrets. Hamsters and ferrets co-inoculated with Delta and Omicron showed full Delta predominance. Moreover, Omicron-related viral RNA was not detectable in ferret nasal washes, nor organs. These results were confirmed by a single infection study of ferrets with the Omicron isolate alone, underlined by a lacking seroconversion of these animals. We conclude that I) Alpha has major fitness advantages over other VOCs in hamsters, II) the ferret model demonstrates high replicating affinity to Delta and III) Omicron VOC leads to a full replication block in ferrets.

P4

Influenza A virus and bacterial co-infection in pigs: What is the role of neutrophil extracellular traps?

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Keywords: Neutrophils, Innate Immunity, Influenza, Pigs, Pasteurellaceae

The *Influenza-A-virus* (IAV) is the causative agent of the flu disease which affects the respiratory tract of humans, poultry and pigs. A co-infection with pathogenic lung bacteria is common and significantly worsens the course of the disease. Neutrophils are recruited to the site of infection and a key defense mechanism is the formation of neutrophil extracellular traps (NETs). They consist of a DNA backbone spiked with antimicrobial components and can entrap or kill invading pathogens. Since degraded NETs provide growth factors, an enhanced growth of bacteria from the familiy *Pasteurellaceae* was observed. The aim of this project is to investigate, if IAV can induce NETs, and thereby initiate the growth of bacterial lung pathogens. Bronchoalveolar lavage fluid (BALF) from IAV positive and negative pigs was biochemically and microbiologically characterized and their influence on bacteria, neutrophils and the host-pathogen interaction was investigated. *Actinobacillus pleuropneumoniae* (*A.pp*), *Glaesserella parasuis* and *Streptococcus suis* show an increased growth in medium supplemented with BALF from IAV diseased pigs naturally containing growth enhancing substances. The growth of *A.pp.* was further enhanced by adding neutrophils from healthy donors *in vitro*, indicating a positive effect of neutrophils on lung bacteria in presence of BALF. IAV was isolated from BALF samples and used in NET induction assays with blood-derived neutrophils. However, preliminary results indicate that IAV does not induce NETs in porcine neutrophils. In conclusion, our data show that factors released from neutrophils may indeed contribute to the severity of a bacterial co-infection during IAV infection in pigs.

P5

Adherence of Streptococcus equi ssp. zooepidemicus to primary cardiac endothelial cells under flow

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Keywords: Streptococcus equi ssp. zooepidemicus*, microfluidic pumpsystem, Human cardiac microvascular endothelial cells, Transposon directed insertion sequencing*

Streptococcus equi ssp. zooepidemicus (SEZ) is a zoonotic pathogen that infects mainly horses and is additionally associated with endocarditis, meningitis, and respiratory diseases in humans. SEZ can adhere to human cells, but underlying pathomechanisms are not yet clarified. Assuming that systemic infections require multifactorial interactions with the cell surface, we aim to identify the bacterial adherence factors, which mediate bacterial cell attachment during an endocarditis infection. Primary human cardiac endothelial cells (HCMEC) serve as model cells to analyse SEZ adherence to the vasculature inducing heart infection. We performed cell culture infection analyses and microscopically quantified SEZ-attachment to HCMEC at various incubation times and bacterial multiplicity of infections (MOI). In order to mimic the altered shear force conditions during heart valve infection, we established a microfluidic pump system that enables the culture of cells under a defined medium flow. In this system, a pneumatic pump presses medium over a confluently grown HCMEC layer, which has been seeded onto specialized microslides allowing microscopic visualization. Immuno fluorescence staining confirmed SEZ adherence to HCMEC in a standardized cell culture infection and at shear forces of 5 and 10 dyn/cm2. In addition, a surface expression profile of selected endothelial receptors of primary HCMEC in static cell culture and after 48 h of flow cultivation was determined by flow cytometry. At later project stages, a Transposon library will be used to identify new bacterial adherence factors comparing the sequences of the bacterial in and output pools via Transposon directed insertion sequencing (TraDIS).

P6

Infection with pathogenic hantaviruses changes the kidney proteome profile

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Keywords: orthohantavirus, mesangial cell, kidney, acute kidney injury, proteome

Acute kidney injury (AKI) is a hallmark of several zoonotic infections. Renal mesangial represent secretory cells and play a key role in the maintenance of normal kidney state. In infected kidneys mesangial cells may induce soluble mediators that induce or counteract direct effects of infection. Eurasian orthohantaviruses such as Puumala virus (PUUV) cause AKI and characterization of the urinary expression profile in infected patients and in in vitro infected renal cells may provide useful insights in the mechanism of AKI in infectious diseases.

We examined the urinary proteome profile of eleven patients suffering from PUUV-induced AKI by analyzing the relative expression level of 38 marker proteins of renal damage compared to a healthy age- and gender-matched control group. To investigate the underlying mechanisms of an altered urinary expression profile, we explored the role of human renal mesangial cells (HRMCs) by in vitro infection studies with pathogenic PUUV and non-pathogenic Tula virus (TULV).

PUUV replicated efficiently in HRMCs and infectious particles were released. In contrast, only few cells were susceptible to TULV and initial infection was abortive. The infection with PUUV resulted in massive changes of the HRMC proteome profile. Cytokines, adhesion molecules and markers of renal damage demonstrated altered expression levels. The comparison with the proteome profile of urinary samples derived from patients revealed strong similarities in the deregulation of proteins. Our findings identify mesangial cells as target cells of viral infection resulting in the induction of a local renal inflammasome that may directly contribute to infection-induced AKI.

Novel Methods, NGS, Genetics N1 – N3

N1

Phenotypic and Genotypic Characterisation of *C. perfringens* isolates from Dairy Cows with a Pathological Puerperium

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Keywords: Clostridium perfringens; dairy cows; metritis; uterine infection; puerperium

Clostridium perfringens (*C. perfringens*) forms part of the intestinal microbiome, but is also a known pathogen in histotoxic infections. The significance of the pathogen as a cause of uterine infections in cattle has been little studied so far. Here, we analyzed the association between a pathological puerperium in cattle and the detection of *C. perfringens* in a prospective longitudinal study. *Clostridium perfringens* were only found in vaginal and uterine samples of diseased cattle, and were absent in healthy controls. Isolates (n = 21) were tested for the production of major toxins (alpha-, beta, epsilon-toxin) by ELISA and for the potential of production of major (alpha-, beta-, iota-toxin) and minor toxins (beta2 toxin) by PCR. Furthermore, antimicrobial susceptibility was also tested phenotypically by microdilution. Despite the frequent use of tetracycline treatment in cows suffering from puerperal disorders, no isolate showed phenotypic tetracycline resistance. Most isolates did not release major amounts of toxin. The strict association of *C. perfringens* with puerperal disease, together with the absence of major toxins might hint towards a major role of other or unknown clostridial virulence factors in uterine disease.

N2

CRISPR-mediated genome editing as a novel method for functional genome analysis of Rhipicephalus microplus ticks

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Keywords: CRISPR-Cas9, RNAi, Rhipicephalus microplus, distalless

Currently, gene silencing by RNA interference (RNAi) is the most widely used tool to examine tick gene function. Despite its many advantages, RNAi does have some limitations as it is for instance not easily applicable all tick life stages, its knockdown effect is transient and complete gene silencing is rarely achieved. CRISPR-Cas9 based gene editing has the potential to overcome many of these disadvantages.

We examined the possibility of inducing CRISPR-Cas9 based gene editing in Rhipicephalus microplus ticks by delivery of the CRISPR/Cas9 ribonucleoprotein complex (RNP), by injection in engorged females followed by electroporation. Successful CRISPR-Cas9 based alteration of the dll gene through the non-homology end joining (NHEJ) pathway is expected to create frameshifts mutations in the target gene causing aberrant limb development.

Combinations of the Cas9 protein with single sgRNAs or sgRNA mixtures were injected in groups of engorged R. microplus females. These groups were exposed to different electroporation conditions and subsequently allowed to oviposit at 27°C and 80% relative humidity. Larvae that hatched were screened phenotypically under a stereomicroscope. DNA was extracted from aberrant larvae and subjected to PCR for dll sequence analysis.

A proportion of the larvae showed aberrant phenotypes such as missing or malformed legs that were not found in the control groups. DNA sequencing confirmed mosaic mutations including insertions and deletions at the expected cutting sites. These results demonstrated that CRISPR-mediated genome editing can be performed by the injection of Cas9 protein with sgRNAs in engorged female ticks followed by electroporation.

N3

Acinetobacter spp. as a source of CRESS DNA in food of animal origin

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Keywords: CRESS DNA, BMMF, Acinetobacter spp., milk

Circular rep-encoding single-strand (CRESS) DNA viruses and molecules similar to them are discussed to be involved in various forms of human cancer and neurodegenerative disease. The detection of these molecules in food of animal origin raises the question about their zoonotic potential and possible sources in the context of entry prevention.

Bovine Meat and Milk Factors (BMMF) are such CRESS DNA molecules. Due to their high diversity, they cannot always be clearly assigned to a taxonomic group. However, their ancestry from bacterial and archaeal plasmids seems certain. Sequence homologies in Genbank entries are observed particularly to plasmids of *Acinetobacter* spp. These microorganisms are strictly aerobic, gram-negative coccoid rod-shaped bacteria which occur ubiquitously.

In our studies, samples of milk from different farms in Schleswig-Holstein, delivered to dairy companies, were analysed for the presence of *Acinetobacter* spp. and BMMF by microbiological and molecular biological methods. Bacteria were detected by culturing on selective agar and confirmed by Maldi-TOF-MS analysis. *Acinetobacter* cultures were analysed with PCR, after a previous rolling circle amplification (RCA), for the presence of the *rep*-gene of BMMF. This procedure was also performed directly from the milk samples. The results of subsequent gene sequencing showed high similarity of the sequences to Genbank entries of *Acinetobacter* spp. as well as to those designated as Bovine Meat and Milk Factors. Some *rep*-gene sequences in extracts of milk and pure culture were homologous, others were not, indicating that *Acinetobacter* is only one of several possible entry factors.

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