# Table of Contents

Welcome Note of the German Research Platform for Zoonoses ..... 2
Welcome Note of the Federal Government ..... 3
Program Zoonoses 2019 - International Symposium on Zoonoses Research ..... 5
General information ..... 14
Floor Plan ..... 18
About the German Research Platform for Zoonoses ..... 19
Oral Presentations ..... 20
Plenary Session I: Keynotes ..... 21
Session 1: Innate and Adaptive Immune Response ..... 24
Session 2: Public Health ..... 31
Session 3: New and Re-Emerging Zoonotic Diseases ..... 38
Session 4: Diagnostics and NGS ..... 45
Session 5: Epidemiology and Ecology of zoonotic infections I ..... 52
Session 6: Epidemiology and Ecology of zoonotic infections II ..... 59
Session 7: Novel Methods ..... 66
Plenary Session II ..... 73
Session 8: Pathogen-Cell Interaction ..... 76
Session 9: New and Re-Emerging Zoonotic Diseases II ..... 83
Session 10: Antimicrobial use and resistance ..... 90
Poster Presentations ..... 100
Poster Presentations Innate and Adaptive Immune Response ..... 101
Poster Presentations Publlic Health ..... 112
Poster Presentations New and Re-Emerging Zoonotic Diseases ..... 130
Poster Presentations Diagnostics ..... 162
Poster Presentations Epidemiology and Ecology of Zoonotic Infections ..... 173
Poster Presentations Novel Methods ..... 196
Poster Presentations Pathogen-Cell Interaction ..... 206
Poster Presentations Antimicrobial use and resistance ..... 228
Poster Presentations Parasitic Zoonoses ..... 256
Poster Presentations Next Generation Sequencing ..... 266
Participants ..... 271
Personal notes ..... 288

## Welcome Note of the German Research Platform for Zoonoses

Dear friends and colleagues,

We are delighted to welcome you to the "**Zoonoses 2019 – International Symposium on Zoonoses Research"** organized by the German Research Platform for Zoonoses in cooperation with the Research Network of Zoonotic Infectious Diseases.

In addition to the new title of the event, acknowledging our increasingly international audience, the structure of the German Research Platform for Zoonoses has slightly changed. With the change of the Berlin office site from TMF to Charité Berlin a new third partner site is introduced. This not only adds a proficient new site to the established offices at the Isle of Riems and Münster, but also ensures an intense cooperation with the Research Network of Zoonotic Infectious Diseases. Benefiting from this new structure, the platform is involved in many projects to further support the zoonoses research community in the future. This includes, among other things, a stronger media presence, an expansion of joint activities with the Public Health sector and new funding opportunities for young scientists.

Once more, the symposium serves as THE paramount annual event to bring together the interdisciplinary zoonoses research community. To meet the high demand and the variety of topics, we have put together a high-quality three-day program with 10 different sessions.

At this point, we would like to thank all participants for submitting their contributions, whether poster or lecture, to help shape the program and to contribute to the success of the symposium.

The program will be complemented by 6 expert Keynote Speakers and the traditional Junior Scientist Breakfast on Friday morning, as well as the annual Members Assembly of the German Research Platform for Zoonoses with the election of the Internal Advisory Board.

We wish all participants a fruitful conference with many opportunities to exchange knowledge, build and refresh networks, and to look beyond everyone`s own professional area. We are proud of the lively zoonoses research community and are taking the next step towards **One Health**.

| **Martin H. Groschup** | **Stephan Ludwig** | **Christian Drosten** |
|---|---|---|
| Greifswald, Riems | Münster | Berlin |

## Welcome Note of the Federal Government

Sehr geehrte Damen und Herren,
liebe Teilnehmerinnen und Teilnehmer,

der Weg von der Jahrestagung der „Nationalen Zoonosenplattform" im Jahr 2007 zum diesjährigen „Internationalen Symposium für Zoonosenforschung" war folgerichtig und notwendig.

Bereits bei der Gründung der „Nationalen Forschungsplattform Zoonosen" im Jahr 2006 war den beteiligten Bundesministerien klar, dass die Risiken, die aus zoonotischen Krankheiten erwachsen, enorm sind und sich die Aufgaben der Zoonosenforschung so schnell nicht erschöpfen werden. Jedoch konnte man kaum die Dynamik voraussehen, mit der die Natur in Sachen Zoonosen auf uns einwirkt. Der Blick hat sich von den nationalen Geschehnissen geweitet auf jene, die sich in der Welt zutragen.

Eine Vielzahl hochpathogener Erreger sind Zoonosenerreger. Einige davon kennen wir aus leidvoller Erfahrung im eigenen Land. Mit vielen Zoonosenerregern waren wir jedoch noch nicht in Kontakt. Wir kennen ihre Eigenschaften aus Lehrbüchern - dem „Immunsystem der heimischen Fauna" sind sie gänzlich unbekannt. Gewichtige Faktoren wie der Klimawandel und der internationale Reise- und Warenverkehr lassen die Welt für Erreger schrumpfen. Was heute noch eine Bedrohung in weit entfernen Ländern ist, trifft uns morgen selbst – einschließlich der soziopolitischen und wirtschaftlichen Auswirkungen auf die Gesundheit und Versorgung von Tier und Mensch.

Die Weltgesundheitsorganisation führt im Jahr 2018 auf ihrer „Blueprint-Liste der prioritären Krankheiten" einen heute noch unbekannten und unbestimmten Erreger auf, den sie "Disease X" nennt. Einen heute unbekannten Erreger, der das Potential hat, eine Pandemie auszulösen. Wissenschaftliche Extrapolationen und Voraussagen nützen nichts, wenn der Staat nicht rechtzeitig vorbereitende Maßnahmen ergreift.

Das Thema des diesjährigen Symposiums ist also zukunftsweisend gewählt – auch wenn der Begriff Tropenkrankheiten für Deutschland auf den ersten Blick unwichtig scheint. Aber das Gegenteil ist richtig: So ist mittlerweile die tropische Zeckenart Hyalomma, die das Fleckfieber übertragen kann, in Deutschland zu finden. Die Förderung der Forschung zu der besonderen Gruppe der zoonotischen „neglected tropical diseases", der sogenannten vernachlässigten Tropenkrankheiten, ist ein zentrales Anliegen des Förderkonzepts „Globale Gesundheit im Mittelpunkt der Forschung" des Bundesministeriums für Bildung und Forschung (BMBF). So widmen sich die BMBF-geförderten Forschungsnetzwerke für Gesundheitsinnovationen in Subsahara-Afrika besonders dem Kampf gegen die Filariose und die Zystizerkose. Die Entwicklung neuer und besserer Behandlungsmöglichkeiten für weitere vernachlässigte Tropenkrankheiten, etwa für die afrikanische Schlafkrankheit oder die Leishmaniose, sind Schwerpunkte der BMBF-Förderung von Produktentwicklungspartnerschaften, kurz PDPs. Die Förderung der nationalen Infektionsforschung ist dabei eine wichtige Grundlage für solche internationalen Kooperationen.

Das Thema „Globale Gesundheit" hat auch für das Bundesministerium für Gesundheit (BMG) einen zunehmend hohen Stellenwert. So wurde im Jahr 2016 das Global Health Protection Programme (GHPP) aufgelegt. Unter Koordination von sechs Institutionen in Deutschland und zahlreichen Partnerinstitutionen in bis zu 40 Ländern – vorwiegend in Afrika – werden mit gezielten Maßnahmen Länder weltweit bei der Prävention und Bekämpfung von Krankheitsausbrüchen unterstützt. Dadurch kann auch ein Beitrag zur Bekämpfung der vernachlässigten Tropenkrankheiten geleistet werden. Auf nationaler Ebene fördert das BMG mit dem Bernhard-Nocht-Institut eine der wichtigsten Einrichtung für Forschung, Versorgung und Lehre auf dem Gebiet von Tropenkrankheiten. Das BNITM beforscht viele

der vernachlässigten Tropenkrankheiten, sowohl im Bereich Grundlagen, Therapieforschung als auch der Entwicklung neuer Diagnostik.

Das Bundesministerium für Ernährung und Landwirtschaft (BMEL) fördert nunmehr in einer zweiten Förderperiode mehrere wissenschaftliche Projekte zu Vektoren, die in der Lage sind, „exotische" Zoonosenerreger zu übertragen. Hierzu zählen der „Mückenatlas" und breite Forschungen zu Steckmücken und deren Vektorkompetenz in Deutschland. Wir wissen nun, dass sich die Asiatische Tigermücke, *Aedes albopictus*, in Deutschland heimisch fühlt. Auch ist davon auszugehen, dass das West-Nil-Fieber-Virus (WNV) in Deutschland überwintert hat. Zahlreiche WNV-Fälle wurden bereits früh in diesem Jahr bei Vögeln und auch beim Pferd bestätigt. Es ist nur eine Frage der Zeit, bis auch erste Fälle beim Menschen auftreten werden.

Die Internationalisierung der Forschung und der damit verbundenen Forschungsfragen steht im BMEL an bedeutender Stelle – zum Schutz der heimischen Tierpopulationen vor Zoonosen.

Auch der Geschäftsbereich des Bundesministeriums der Verteidigung (BMVg) ist mit seinem Institut für Mikrobiologie der Bundeswehr an Projekten des Zoonosen-Netzwerks beteiligt und wirkt in den beiden Forschungsverbünden zu FSME und Q-Fieber mit.

Da sieben der neun Erreger des sogenannten „Dirty Dozen" zoonotische Erreger sind, haben diese Erreger auch eine zentrale Bedeutung für den Medizinischen B-Schutz. Aus diesem Grund hat die Bundeswehr dieses Jahr einen eigenen Forschungs-Schwerpunkt zu zoonotischen Erkrankungen mit wehrmedizinischer Relevanz gebildet.

Das Institut für Mikrobiologie der Bundeswehr war das erste Institut weltweit, das in einem militärisch-operativen Kontext die Technologie des Next-Generation-Sequenzing im Feldeinsatz erfolgreich evaluieren und damit seine bestehenden Diagnostikfähigkeiten nennenswert erweitern konnte. Lag der Fokus bei dieser Technologie bislang im Bereich der Bakteriologie, soll er künftig in den Bereich der Virologie mit dem Schwerpunkt auf zoonotische Erreger verlagert werden. Aufgrund der zunehmenden einsatzbezogenen Aufgaben hat dieser Ansatz eine wichtige Bedeutung auch für die Bundeswehr.

Für alle Ressorts gilt daher, wenn auch unter verschiedenen Voraussetzungen: Zoonosen müssen dort bekämpft werden, wo sie auftreten!

Diese Feststellung berührt mehrere Aspekte. Im internationalen Rahmen sollten Expertise und Material bereitgestellt werden, sofern dies gewünscht ist. Eine rasche Tilgung am Ort des Geschehens ist nicht nur unabdingbar zum Schutz von Leben und Gesundheit von Tier und Mensch, sondern trägt zur Stabilisierung betroffener Regionen bei. Eine Bekämpfung vor Ort ermöglicht auch Wissenschaftlern der verschiedenen Fachrichtungen, bei der gemeinsamen Arbeit wichtige Erfahrungen für einen möglichen Ausbruch der Krankheit im eigenen Land zu sammeln. All dies trägt ganz wesentlich zur Vorbereitung auf mögliche Ausbrüche im eigenen Land bei und demonstriert gleichzeitig die erfolgreiche Umsetzung von „One Health", dem Konzept, dem sich alle hier Anwesenden verpflichtet fühlen.

In diesem Sinne wünsche ich Ihnen eine erfolgreiche, diskussions- und ideenreiche Veranstaltung!

Bundesministerium für Bildung und Forschung - Federal Ministry of Education and Research (BMBF)

Bundesministerium für Gesundheit - Federal Ministry of Health (BMG)

Bundesministerium der Verteidigung - Federal Ministry of Defense (BMVg)

Bundesministerium für Ernährung und Landwirtschaft - Federal Ministry of Food and Agriculture (BMEL)

# Program Zoonoses 2019 - International Symposium on Zoonoses Research

Joint meeting of the National Research Platform for Zoonoses and the Research Network of Zoonotic Infectious Diseases

 *= contributions from the Research Network of Zoonotic Infectious Diseases*

**Wednesday, October 16, 2019**

**14:00 Registration Opens (Poster Mounting)**

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**15:00 – 17:00 Plenary Session I: Keynotes (Room Ballsaal)**

Language: English/ German
Chair: *Christian Drosten*

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15:00 **Opening Remarks: Joint meeting of the German Research Platform for Zoonoses**
**and the Research Network of Zoonotic Infectious Diseases**
Martin H. Groschup (Friedrich-Loeffler-Institute, Greifswald – Isle of Riems) & Christian Drosten (Charité Berlin)

**Welcome Note of the Federal Government**
Dietrich Rassow (Federal Ministry of Food and Agriculture)

15:30 **Keynote I:**
**A massive West Nile virus epizootic in Germany, 2018/19**
Martin H. Groschup, Friedrich-Loeffler-Institute, Greifswald – Isle of Riems, Germany

16:15 **Keynote II:**
**Arboviruses as Neglected Tropical Diseases – Dengue, Zika, Chikungunya**
Thomas Jänisch, Institute of Global Health, Heidelberg University Hospital, Germany

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17:00 Coffee Break

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**17:20 – 18:50 Session 1: Innate and Adaptive Immune Response (Room Ballsaal)**
Chairs: *Michael Mühlebach and Christian Menge*

17:20 **Carvacrol, the major component of oregano oil, alleviates *Campylobacter jejuni* induced acute enterocolitis in a clinical murine model for campylobacteriosis**
**Markus M. Heimesaat**, Soraya Mousavi, Ulrike Escher, Anna-Maria Schmidt, Stefan Bereswill

17:35 **A single amino acid position exchange in the nucleoprotein of the tick transmitted Thogotovirus leads to complete escape from human MxA restriction**
**Jonas Fuchs,** Laura Graf, Alexander Oschwald, Georg Koch

17:50 **Translational studies exploring the lack of immunity against rabies virus infection**
**Corine Geurts van Kessel,** Carmen Embregts, Thijs Kuiken, Marion Koopmans

18:05 **T-lymphocyte homing signatures in human Lassa fever**
**Julia Port,** David Wozniak, Elisa Pallasch, Yemisi Ighodalo, Stephan Günther, Lisa Oestereich, César Muñoz-Fontela

18:20 **Novel therapeutic approach to cure Lassa fever**
**Lisa Oestereich**, Sabrina Bockholt, Elisa Pallasch, Stephan Günther

18:35 ***Coxiella burnetii* escapes cellular self-defense of infected NK cells**
**Svea Matthiesen,** Kati Franzke, Michael R. Knittler, Rico Jahnke

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**17:20 – 18:50 Session 2: Public Health**
Chairs: *Susanne Kutzora and Stefan Brockmann*

17:20 **ts-mutants of porcine influenza viruses: Pathway to live-attenuated vaccines in pigs?**
**Annika Graaf**, Fabian Deutskens, Dinah Henritzi, Anja Petrov, Philipp Petric, Martin Schwemmle, Martin Beer, Timm Harder

17:35 **Sensibilisation of general practicioners towards imported arboviral infections in the light of established populations of *Aedes albopictus* in Baden-Württemberg 2019**
**Hannah Höglund-Braun,** Christiane Wagner-Wiening

17:50 **Tick-borne encephalitis virus isolated from natural foci causing TBE vaccine breakthroughs**
**Gerhard Dobler,** Lidia Chitimia-Dobler, Malena Bestehorn-Willmann

18:05 **Molecular epidemiology of hantavirus disease in Germany**
Mirko Faber, Detlev H. Kruger, Klaus Stark, Jörg Hofmann, **Sabrina Weiss**

18:20 **Prevention of zoonoses by using traditional and social media for health communication by local public health authorities (LPHA) in Germany**
**Sebastian Kleele,** Christina Princk, Marion Müller, Maren Mylius, Johannes Dreesman, Kerstin Dressel

18:35 **Impact of hygiene- and management measures on the ESBL- and AmpC-colonization of broiler chickens**
**Caroline Robé,** Katrin Daehre, Roswitha Merle, Sophie Fiedler, Christa Ewers, Sebastian Guenther, Uwe Roesler

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18:50 Poster Viewing Session/ Welcome Reception

**Thursday, October 17, 2019**

**08:30** **Registration continued**

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**09:00 – 10:30** **Session 3: New and Re-Emerging Zoonotic Diseases (Room Ballsaal)**
Chairs: *Asisa Volz and Christian Drosten*

09:00 **Genetic diversity of *Treponema pallidum* subsp. *pertenue* infecting wild non-human primates in Taï National Park (TNP), Cote d'Ivoire**
**Benjamin Mubemba,** Jan Gogarten , Ariane Düx , Markus Ulrich, Kamilla Pléh, Fabian Leendertz, Sebastien Calvignac-Spencer

09:15 **Development and characterization of small animal models for variegated squirrel bornavirus 1 (VSBV-1)**
**Kore Schlottau,** Daniel Nobach, Christiane Herden, Martin Beer, Donata Hoffmann

09:30 **Fatal human variegated squirrel bornavirus 1 encephalitis is characterized by immunopathological changes**
**Dennis Tappe**, Jonas Schmidt-Chanasit, Jessica Rauch, Christiane Herden

09:45 **Isolation and characterization of Puumala orthohantavirus from Germany**
**Florian Binder,** Marion Saathoff, Jona Freise, Rainer G. Ulrich

10:00 **In vivo evaluation of a MVA based ZikV antigen library in a mouse model for ZikV disease**
**Jan Schwarz,** Dominik Forster, Gerd Sutter, Asisa Volz

10:15 **New "omics"-insights into the genome evolution and virulence potential of the emerging group of marine Brucella species**
**Dirk Hofreuter,** Holger Brendebach, Sandro Andreotti, Kerstin Neubert, Boyke Bunk, Matthias Flor, Josephine Grützke, Cathrin Spröer, David Fretin, Jörg Overmann, Sascha Al Dahouk

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**09:00 – 10:30** **Session 4: Diagnostics and NGS (Room Zehlendorf)**
Chairs: *Victor Corman and Martin Beer*

09:00 **Fast differentiation of Enterobacteriaceae using Raman Spectroscopy**
**Amir Nakar,** Karina Weber, Sascha D. Braun, Ralf Ehricht, Petra Rösch, Jürgen Popp

09:15 **Influence of infection route on neuropathology of tick-borne encephalitis virus in a murine infection model**
**Mathias Boelke,** Christina Puff, Fanny Naccache, Frederic Gusmag, Katrin Liebig, Sonja T. Jesse, Andreas Beineke, Gerhard Dobler, Ab D.M.E. Osterhaus, Wolfgang Baumgärtner, Claudia Schulz and Stefanie C. Becker

09:30 **Rabies virus in Namibian Kudus – Exploring the Genetic Diversity of Lyssaviruses**
**M. Sten Calvelage,** Conrad Freuling, Thomas Müller, Martin Beer, Dirk Höper

09:45 **Concordance between genomic typing methods can simplify data exchange for surveillance and control of infectious diseases**
**Stefanie Lüth,** Carlus Deneke, Sylvia Kleta, Sascha Al Dahouk

10:00 **Genome-Wide Identification of Genomic Determinants for Host Restriction in *Campylobacter jejuni***
**Lenard Epping**, Rosario M. Piro, Marie-Theres Knüver, Maria Borowiak, Charlotte Huber, Andrea Thürmer, Burkhard Malorny, Kerstin Stingl, Angelika Fruth, Lothar H. Wieler, Torsten Semmler

10:15 **Identification of host-associated genomic determinants in *Escherichia coli***
**Sumeet Kumar Tiwari**, Boas Van der Putten Trung Nguyen V., Martin Bootsma, Roberto La Ragione, Sebastien Matamoros, Hoa Ngo T., Christian Berens, Joy Leng, Julio Álvarez, Dominique Jolivet, Marta Ferrandis-Vila, Jenny Ritchie, Angelika Fruth, Stefan Schwarz, Lucas Domínguez, María Ugarte-Ruiz, Astrid Bethe, Lothar H. Wieler, Christian Menge, Amanda Fivian-Hughes, Constance Schultsz, Torsten Semmler

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**09:00 – 10:30 Session 5: Epidemiology and Ecology of zoonotic infections I (Room Steglitz)**
Chairs: *Jonas Schmidt-Chanasit and Ard Nijhof*

09:00 **Are ESBL- producing *E. coli* spread in the environment by manure application, soil cultivation and wind erosion?**
**Paul Siller,** Katrin Dähre, Uwe Rösler

09:15 **Cross-Protection of Dengue Virus Infection against Congenital Zika Syndrome, Northeastern Brazil**
Celia Pedroso, **Carlo Fischer**, Marie Feldmann, Eduardo Martins netto, Beate M. Kümmerer, Jan Felix Drexler

09:30 **Tropical rainforest flies carrying pathogens form stable associations with social non-human primates**
**Jan Frederik Gogarten,** Ariane Düx, Benjamin Mubemba, Kamilla Pléh, Constanze Hoffmann, Alexander Mielke, Jonathan Müller-Tiburtius, Andreas Sachse, Roman Wittig, Sébastien Calvignac-Spencer, Fabian Leendertz

09:45 **Does cold winter really limit the dengue vector *Aedes aegypti* in Europe? – A meta analysis and experimental evaluation of the cold tolerance of *Ae. aegypti* versus *Ae. albopictus* eggs**
**Isabelle Kramer,** Christian Scherer, Aljoscha Kreß, Meghnath Dhimal, David A. Groneberg, Bodo Ahrens, Ulrich Kuch, Parbati Phuyal, Doris Klingelhöfer, Ruth Müller

10:00 **West West Nile virus in Germany: vector competence of native *Culex pipiens* and *Aedes albopictus***
**Cora Marielle Holicki**, Ute Ziegler, Helge Kampen, Doreen Werner, Cornelia Silaghi, Martin H. Groschup, Ana Vasić

10:15 **Evaluation of the vector competence of ticks for *Coxiella burnetii* using an artificial feeding system**
**Sophia Körner,** Gustavo R. Makert, Katja Mertens-Scholz, Klaus Henning, Martin Pfeffer, Sebastian Ulbert

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10:30 Coffee Break and Poster Viewing (even poster numbers)

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**11:00 – 12:30 Session 6: Epidemiology and Ecology of zoonotic infections II (Room Ballsaal)**
Chairs: *Ruth Müller and Rainer Ulrich*

11:00 **Epidemiology of Leptospirosis in Uganda - Starting from Scratch**
**Anou Dreyfus**, Lordrick Alinaitwe, Kathryn Allan, Sabrina Rodriguez-Campos, Paul Torgerson, Jonathan W Dyal, David Boulware, Raewynne Pearson, Terence Odoch, Charles Kajura, Steven Kakooza, Katharine Pelican, Dominic Travis, Michael Mahero, Christina Faust, Edridah M Tukahebwa, Valentine Jaquier, Amanuel Tsegay, Lawrence Mugisha, Clovice Kankya

11:15 **A multitaxa study on virus diversity and abundance in habitats differing in their level of disturbance reveals virus-host-specific prevalence patterns**
**Kyra Hermanns,** Florian Zirkel, Sandra Junglen

11:30 **Anthroponotic transmission of *Cryptosporidium spp*. in rural sub Saharan Africa: a one health study**
**Daniel Eibach**, Ralf Krumkamp, Simone Caccio, Akim Adegnika, John Amuasi, John Lusingu, Raphael Rakotozandrindrainy, Jürgen May

11:45 **Tick-borne encephalitis: First results of intensified surveillance in Bavaria and Baden-Württemberg (TBENAGER)**
**Teresa M. Kreusch**, Merle M. Böhmer, Christiane Wagner-Wiening, Ole Wichmann, Wiebke Hellenbrand

12:00 **Citizen science[3]: surveillance of Usutu virus activity in Germany**
**Renke Lühken**, Daniel Cadar, Norbert Becker, Stefan Bosch, Lars Lachmann, Jonas Schmidt-Chanasit

12:15 **Cluster Analysis with German Antimicrobial Resistance (AMR) Data for Humans and Food-producing Animals**
**Beneditta Suwono**, Bernd-Alois Tenhagen, Tim Eckmanns, Heike Kaspar

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**11:00 – 12:30 Session 7: Novel Methods (Room Steglitz)**
Chairs: *Imke Steffen and Sascha Al-Dahouk*

11:00 **An antique origin of measles**
**Ariane Düx**, Sebastian Lequime, Livia Patrono, Baptiste Prepoint, Markus Ulrich, Jasmin Schlotterbeck, Jan Gogarten, Kevin Merkel, Annette Mankertz, Navena Widulin, Fabian Leendertz, Thomas Schanlke, Philippe Lemey, Sébastien Calvignac-Spencer

11:15 **Rabies Virus Cell Tropism in 3D – comparison of attenuated, lab and field strains**
**Madlin Potratz,** Luca Zaeck, Stefan Finke

11:30 **Three-dimensional co-cultivation of cells from different species and tissue origin in a novel bioprinted array as a diagnostic tool for virus detection**
**Robert Koban,** Markus Neumann, Franziska Schwarz, Tobias Lam, Lutz Kloke, Heinz Ellerbrok

11:45 **Differential efficacy of novel antiviral substances against Cowpox virus (CPXV) in 3D and monolayer cell culture**
**Markus Neumann,** Robert Koban, Philopp Nelson, Heinz Ellerbrok

12:00 **A specialized thin agar method (STAM) to study electric signaling in bacterial biofilms**
**Julia Assmann**, Silvio Bürge, Christoph Schaudinn, Michael Laue, Birgit Walther

12:15 **An in vitro system to study heteroresistance and metabolic host interaction on mature tissue cysts of *Toxoplasma gondii***
**Céline Christiansen,** Jana Scholz, Frank Seeber, Michael Laue, Martin Blume

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12:30 Lunch & Poster Viewing
12.30 - 13.30 even poster numbers
13.30 - 14.30 odd poster numbers

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**14:30 – 16:00 Plenary Session II (Keynotes) (Room Ballsaal)**
Chair: *Martin H. Groschup*

14:30 **Keynote III:**
**Animal tuberculosis: the challenge of controlling a global multi-host infection**
Christian Gortázar Schmidt, Institute for Game and Wildlife Research, Universidad de Castilla, Spain

15:15 **Keynote IV:**
**Situation of leishmaniosis in Europe from humans to wildlife**
Laia Solana-Gallego, Department of Animal Medicine and Surgery, Autonomous University of Barcelona, Spain

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16:00 Coffee Break

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**17:00 – 19:00 General Assembly National Research Platform for Zoonoses with the Election of Internal Advisory Board (Room Ballsaal)**
Language: German
Chairs: *Stephan Ludwig, Martin H. Groschup, Christian Drosten*

Wahl des Internen Beirats
Bericht über das vergangene Jahr

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**19:00 – 20:00** Poster Viewing Session (odd poster numbers) + Casual Reception

**20:00 Social Dinner (Room Ballsaal)**

**Friday, October 18, 2019**

**07:30 – 09:00 Young Scientist Breakfast**

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**09:30 – 11:00 Session 8: Pathogen-Cell Interaction (Room Zehlendorf)**

09:30 **Immunogenicity and fetoprotective capacity of recombinant MVA Virus expressing Zika Virus E and prM/M proteins in a pregnant mouse model**
**Dominik Forster,** Jan Hendrik Schwarz, Gerd Sutter, Asisa Volz

09:45 **Impact of polymorphisms in the MERS-coronavirus spike protein and its receptor DPP4 on viral entry into host cells**
**Hannah Kleine-Weber,** Simon Schröder, Nadine Krüger, Khaled R. Alkharsah, Marcel A. Müller, Christian Drosten, Stefan Pöhlmann, Markus Hoffmann

10:00 **A single amino acid substitution in the Ebola virus glycoprotein reduces the dependence of host cell entry on cellular cysteine proteases**
**Markus Hoffmann,** Svenja Kaufmann, Carina Fischer, Wiebke Maurer, Stefan Pöhlmann

10:15 **Enhanced replicative capacity and anti-immune activity of epidemic recombinant MERS-Coronavirus**
**Simon Schroeder,** Christian Drosten, Marcel Müller, Victor Corman, Doreen Muth, Ronald Dijkman, Thorsten Wolff, Christin Mache

10:30 **Structure of a functional cap-binding domain in Rift Valley fever virus L protein**
Nadja Gogrefe, Sophia Reindl, Stephan Günther, **Maria Rosenthal, Silke Olschewski**

10:45 **Single cell assay for DNA uptake in *Campylobacter jejuni* - a tool for monitoring the adaptive potential of the food-borne pathogen**
**Julia Golz**, Kerstin Stingl

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**09:30 – 11:00 Session 9: New and Re-Emerging Zoonotic Diseases II (Room Steglitz)**

09:30 **The cowpox virus reservoir – are host-species specific strains the key?**
**Saskia Weber,** Kathrin Jeske, Florian Pfaff, Rainer G. Ulrich, Martin Beer, Donata Hoffmann

09:45 **Phenotypes of MERS-CoV infections in human lung explant cultures**
**Christin Mache,** Simon Schröder, Diana Fatykhova, Andreas Hocke, Stefan Hippenstiel, Christian Drosten, Thorsten Wolff

10:00 **Establishment of a reverse genetics system for the cell-culture-adapted hepatitis E virus genotype 3c-strain 47832c**
**Johannes Scholz**, Alexander Falkenhagen, Christine Bächlein, Reimar Johne

10:15 **Identification of animal reservoirs of Fort Sherman virus, a New World zoonotic orthobunyavirus**
**Edmilson F. de Oliveira Filho**, Roberto Franke, Ianei Carneiro, Jan Felix Drexler, Carlo Fischer

10:30 **Mammalian hepatitis delta virus without hepadnavirus coinfection in the neotropical rodent *Proechimys semispinosus***

**Sofia Paraskevopoulou,** Fabian Pirzer, Julian Schmid, Victor Max Corman, Nora Goldmann, Simon Schroeder, Lina Theresa Gottula, Andrea Rasche, Doreen Muth, Jan Felix Drexler, Alexander Christoph Heni, Georg Joachim Eibner, Rachel A. Page, Terry C. Jones, Marcel Alexander Müller, Dieter Glebe, Simone Sommer, Christian Drosten

10:45 **Arenavirus persistence in *Mastomys natalensis*, the natural rodent reservoir of Lassa virus**
**Chris Hoffmann**, Elisa Pallasch, Sabrina Bockholt, David Wozniak, Jonas Müller, Lisa Oestereich, Stephan Günther

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**09:30 – 11:00 Session 10: Antimicrobial use and resistance (Room Ballsaal)**

09:30 **Vitamin C alleviates *Campylobacter jejuni* induced acute enterocolitis in a clinical model for acute campylobacteriosis**
**Stefan Bereswill**

09:45 **Characteristics of a P1-/P7-like prophage mediating transmission of an incorporated transposon comprising a blaCTX-M-15 resistance gene**
**Jens Andre Hammerl,** Claudia Jäckel, Valerie Osieka, Silvia Schmoger, Claudia Szentiks, Annemarie Käsbohrer

10:00 **Impact of two antibiotic regimes on composition and diversity of gut microbiomes in horses with colic surgery – a comparative analysis**
**Anne Kauter,** Antina Lübke-Becker, Dania Kanapin, Sabita Stöckle, Lennard Epping, Torsten Semmler, Lothar H. Wieler, Heidrun Gehlen, Birgit Walther

10:15 **Bacterial adaptation to surfactant antimicrobials**
**Olga Makarova,** Phil Ferguson, Paul Johnston, A. James Mason, Jens Rolff, Uwe Roesler

10:30 **The mode of action of *T. gondii* tissue cyst inhibitors**
**Deborah Maus,** Martin Blume, Elyzana Putrianti, Jens Pikkemaat

10:45 **Antimicrobial usage in German cattle farms - which groups of active ingredients are used?**
**Katharina Hommerich**, Maria Hartmann, Svetlana Kasabova, Annemarie Käsbohrer, Lothar Kreienbrock

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11:00 Coffee Break & Poster Viewing (all poster numbers)

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**11:30 – 12:15 Plenary Session III: Keynotes**
**(Room Ballsaal)**
Language: English
Chair: *Stephan Ludwig*

**11:30 Keynote V:**
**Human filarial infections – a group of NTDs with a zoonotic relative of increasing importance**
Achim Hörauf, Bonn University Medical Center, Germany

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12:15 Lunch & Poster Viewing (all poster numbers)

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**13:15 – 15:00 Plenary Session III (continued): Keynotes**
**(Room Ballsaal)**
Language: English
Chair: *Stephan Ludwig*

13:15 **Keynote VI:**
**Nipah Virus: An Emerging Zoonosis and Global Health Threat**
Jonathan H. Epstein, EcoHealth Alliance, New York, USA

14:00 **Poster Awards and Farewell**

15:00 **End of Meeting**

# General information

**Date and Venue**
October 16-18, 2019

Hotel Steglitz International
Albrechtstraße 2, 12165 Berlin
www.si-hotel.com

**Conference Languages**
The official conference languages are English and German.

**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
Christian Drosten
Ilia Semmler

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**Poster Prize**
All participants of the meeting are encouraged to elect the three best posters presented by Junior Scientists at the symposium. Junior Scientist posters are marked with a colored dot in the right hand corner. Ballots for the election will be handed out to each participant during the registration process. Each participants has two votes. All ballots have to be returned to the registration desk by Friday 9:30 am. Ballots handed in after that time will not be counted for the election. The winners will be announced on the last day of the meeting.

**Keynote Speakers**
Thomas Jänisch, Heidelberg, Germany
Achim Hörauf, Bonn, Germany
Jonathan H. Epstein, New York, USA
Martin H. Groschup, Greifswald, Germany
Laia Solana-Gallego, Barcelona, Spain
Christian Gortázar Schmidt, Castilla, Spain

**Young Scientists Breakfast**
The Young Scientists Breakfast is going to take place at the restaurant of the Hotel Steglitz Int. on Friday, October 18th, at 7:30 am. Junior Scientists can register for the event at the registration desk. Please note that the number of available places is limited.

**Lunch Set-up**
Due to the capacity of the venue premises, lunch will be served in two consecutive shifts. Please exercise some patience while seating yourself accordingly.

**Continuous Medical Education & Continuous Veterinary Education**
The International Symposium on Zoonoses Research is registered for CME-points and ATF-Stunden.

**Poster Presentations**
Posters will be presented during all three days of the conference. The posters will be allocated in the rooms Lankwitz, Atrium and Foyer. The schedule for poster presentations is the following:

| | | |
|---|---|---|
| Wednesday, October 16th | 18:50 | all posters |
| Thursday, October 17th | 10:30 – 11:00 | even poster numbers |
| | 12:30 – 13:30 | even poster numbers |
| | 13:30 – 14:30 | odd poster numbers |
| | 19:00 – 20:00 | odd poster numbers |
| Friday, October 18th | 11:00 – 11:30 | all posters |
| | 12:15 – 13:15 | all posters |

Poster presenters will obtain their poster number during registration process and are requested to refer to this booklet and the relevant bulletin on the blackboard to find the poster session and board number assigned to them. Please use the poster board with the designated number. Poster presenters are responsible to remove the posters at the end of the conference.

| Room | Poster numbers | General topic |
|---|---|---|
| Atrium | C01 – C21 | Pathogen-Cell Interactions |
| | P01 – P09 | Parasitic Zoonoses |
| Lankwitz | A01 – A27 | Antimicrobial use and resistance |
| | M01 – M09 | Novel Methods |
| Foyer | D01 – D10 | Diagnosis |
| | E01 – E22 | Epidemiology and Ecology |
| | I01 – I10 | Innate and Adaptive Immune Response |
| | N01 – N31 | New and re-emerging Zoonoses |
| | S01 – S04 | NGS |
| | H01 – H17 | Public Health |

**Oral Presentations**
Oral presentations should be handed in on a common data carrier at the registration desk starting Wednesday, October 16 at 2.00 pm. All session rooms will be equipped with a PC computer and a LCD projector. **Apple computers are not available.** Please make sure that you use either a PowerPoint or a pdf file format.

**Internet Access**
For internet access you are pleased to register at the registration desk. WLAN will be provided without charge.

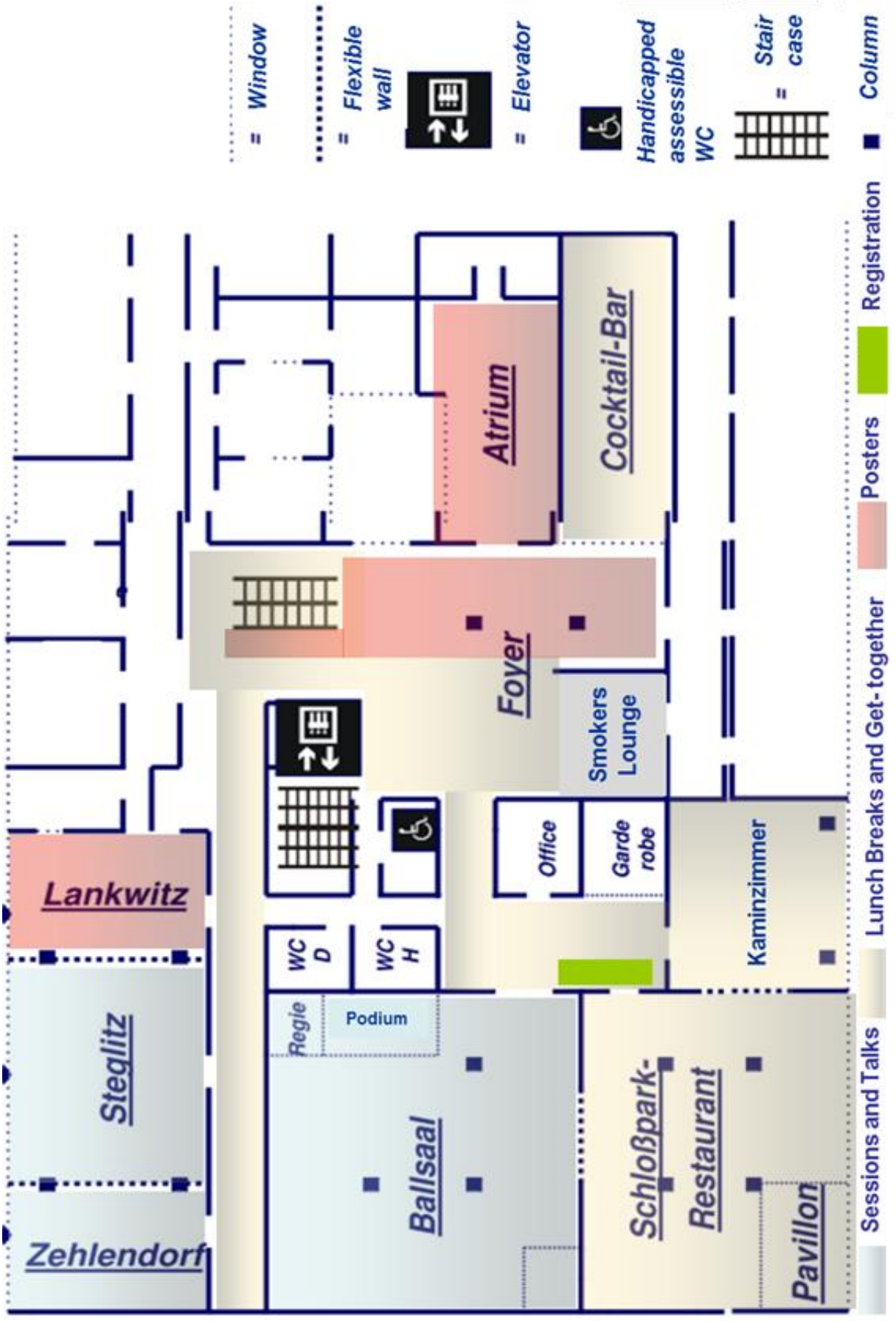
**Funding**
TheInternational Symposium on Zoonoses Research (Zoonoses 2019) is funded by the Federal Ministry of Education and Research.

Sessions of the Research Network of Zoonotic Infectious Diseases are funded by the Federal Ministry of Education and Research.

**Sponsoring**
The National Symposium on Zoonoses Research is kindly supported by:


Please feel free to take a look at the Pfizer booth in the foyer.

## Floor Plan


= Window
= Flexible wall
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Handicapped assessible WC
Stair = case
Column
Registration
Posters
Lunch Breaks and Get-together
Sessions and Talks
Lankwitz
Steglitz
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Regie
Podium
WC D
WC H
Foyer
Atrium
Cocktail-Bar
Smokers Lounge
Office
Garde robe
Kaminzimmer
Schloßpark-Restaurant
Pavillon

## 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 800 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 pilot projects of a cross-sectional nature
- 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 around 350 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.

# Oral Presentations

## Plenary Session I: Keynotes

**October 16, 2019**
**15:00 – 17:00**

**Room: Ballsaal**
**Chair: Christian Drosten**

## A massive West Nile virus epizootic in Germany, 2018/19

Ute Ziegler[1], Jonas Schmidt-Chanasit[2], Martin H. Groschup[1]

[1]Friedrich-Loeffler-Institute, Institute of Novel and Emerging Infectious Diseases (INNT), Greifswald, Germany

[1]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany; German Centre for Infection Research (DZIF), Partner Site Hamburg-Luebeck-Borstel, Greifswald-Insel Riems, Germany
[2]Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany

Central Europe experienced remarkably hot and dry summers in the last two years. These extremely favorable climatic conditions most likely triggered the expansion and the efficient propagation of the zoonotic arthropod-borne West Nile virus (WNV) from Southern/Southeastern to Central Europe. WNV is a mosquito-borne viral pathogen of global importance. Until July 2018, WNV was not detected in Germany. However, first WNV infections were detected in resident wild and aviary birds as well as in equines in Eastern and Southeastern Germany since August 2018. The causative WNV strain belonged to the central European subclade II. Phylogeographic analysis indicated a single introduction event, most likely in 2016 from the Czech Republic. These initial outbreaks were followed in 2019 by an even larger epizootic inflicting a broad variety of wild and aviary bird species (58 fatal cases), horses (15 clinical cases, thereof 2 fatalities) and even two humans (one case of severe encephalitis) (data as of 23.9.19). Phylogenetic data indicate overwintering of the 2018 isolate as well as new virus introductions into Germany. WNV hotspot areas are in Saxony, Saxony-Anhalt, Berlin, and some regions in Brandenburg. Available epidemiological data indicate that an even further spread to more areas in Germany and more animal and human WNV cases/fatalities

## Arboviruses as Neglected Tropical Diseases – Dengue, Zika, Chikungunya

Thomas Jaenisch, MD PhD[1, 2]

[1]Head, NTD Unit, Institute of Global Health, Heidelberg University Hospital
[2]Arbovirus Research Consortium, Department of Epidemiology, Colorado School of Public Health

*Keywords: Arboviruses, Dengue, Chikungunya, Zika, Neglected Tropical Diseases*

The emergence and resurgence of arthropod-borne viruses (arboviruses), among others Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV), constitutes an ongoing challenge to public health and medicine affecting 3.6 billion people at risk worldwide. DENV, CHIKV, and ZIKV are transmitted by the same mosquito species, which is able to take advantage of globalization and urbanization dynamics. If symptomatic, the diseases are characterized by a substantial clinical overlap, which highlights the need for laboratory diagnostics. However, due to cross-reactivity in serological tests, DENV and ZIKV cannot be well distinguished.
During the last decades, DENV caused a silent pandemic, spreading into previously uninfected areas. At least for Africa, DENV can be considered neglected. CHIKV emerged in Latin America for the first time in 2014 and has since spread over the continent, causing a high burden of disability, mainly persisting arthritis. Because of the adaptation to *Aedes albopictus* (the 'Asian Tiger Mosquito'), it currently has the highest emergence potential in Europe. Starting in 2015, ZIKV swept through most of Latin America, but severe complications were mainly reported in the Northeast of Brazil. This imbalance has puzzled many researchers, some of whom believe that the pattern is associated with co-factors, including previous Dengue infections. Public Health authorities have also warned that an introduction of the Asian ZIKV genotype into Africa could potentially lead to a high disease burden.
DENV and CHIKV are formally part of the WHO list of NTDs, whereas ZIKV is listed under the blueprint priority diseases.

## Session 1: Innate and Adaptive Immune Response

**October 16, 2019**
**17:20 – 18:50**

**Room: Ballsaal**
**Chairs: Michael Mühlebach and Christian Menge**

## Carvacrol, the major component of oregano oil, alleviates Campylobacter jejuni induced acute enterocolitis in a clinical murine model for campylobacteriosis

Markus M. Heimesaat, Soraya Mousavi, Ulrike Escher, Anna-Maria Schmidt, Stefan Bereswill

Institute of Microbiology & Infection Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany; PAC-Campylobacter consortium (IP7)

*Keywords: Campylobacteriosis, carvacrol, intestinal and extra-intestinal including systemic anti-inflammatory effects*

Background and objectives: Our recent intestinal metabolomic analyses revealed that distinct plant dervied substances might be involved in mediating colonization resistance against Campylobacter. We here addressed whether peroral application of synthetic carvacrol might be a prophylactic measure for combating C. jejuni induced immunopathology in a clinical model for campylobacteriosis.
Materials and methods: Secondary abiotic IL-$10^{-/-}$ mice were subjected to carvacrol treatment via the drinking water starting four days peroral infection with viable C. jejuni 81-176 strain (day 0).
Results: Six days post C. jejuni infection, carvacrol treated mice developed significantly less severe symptoms as compared to placebo controls. Particularly treated mice further displayed less pronounced apoptotic cell and pro-inflammatory immune responses that were not restricted to the intestinal tract, but could also be observed in extra-intestinal compartments and, remarkably, systemically. Strikingly, intestinal C. jejuni loads of carvacrol treated mice up to two log orders of magnitude lower at day 6 post-infection as compared to mock controls with median fecal burdens of $10^9$ CFU per g.
Conclusion: Due to its potent anti-inflammatory effects in murine infection models, carvacrol represents a promising option for treatment and prophylaxis of Campylobacter infection and colonization in humans and farm animals, respectively.

## A single amino acid position exchange in the nucleoprotein of the tick-transmitted Thogotovirus leads to complete escape from human MxA restriction

Jonas Fuchs, Laura Graf, Alexander Oschwald, Georg Kochs

Institute of Virology, Medical Center, Faculty of Medicine, University Freiburg, Germany

*Keywords: tick transmitted Thogotovirus, zoonotic transmission, Myxovirus resistance protein A, MxA*

Thogotoviruses are tick-transmitted Orthomyxoviruses that primarily cause disease in livestock. However, recent fatal human cases of Thogotovirus (THOV) infections in the US suggest a zoonotic potential. We previously showed that THOV are controlled by the interferon induced human MxA protein in cell culture and in vivo. This antiviral effector binds the viral nucleoprotein (NP), thus efficiently inhibiting viral replication.
We hypothesize that acquiring MxA resistance would qualify THOV to cross the species barrier. To this end, we screened for the effect of human MxA on ten different THOV isolates. By infecting transgenic MxA mice we identified a particular isolate – JOSV- that was replicating to normal titers whereas the prototype isolate (SiAr126) was potently inhibited. We subsequently constructed chimeric NPs between SiAr126 and JOSV which led us to the identification of a single amino acid substitution (R328V) leading to complete MxA escape in vivo and loss of NP recognition by MxA in vitro.
Our analysis identified a yet unknown zoonotic risk of Thogotoviruses and reveals their capacity to overcome species barrier by escaping MxA restriction.

## Translational studies exploring the lack of immunity against rabies virus infection

Embregts, C.W.E., Kuiken, T., Koopmans M., Geurts van Kessel, C.

Department of Viroscience, Erasmus Medical Centre, Rotterdam, the Netherlands

*Keywords: Rabies, germinal centers, humoral immune response*

Rabies encephalitis is caused by infection with a lyssavirus, including rabies virus (RABV). Once clinical signs develop, the outcome is nearly always fatal. Most of the people suffering from Rabies do not develop specific antibody titres. It is not understood why an efficient immune response against RABV is not being mounted upon infection with RABV, whereas innate immune cells will be exposed to the virus at the site of infection. Understanding why an immune response against RABV is not mounted is essential to develop novel treatment strategies against the disease.
We performed post-mortem analysis on various organs from humans and animals that died from RABV infection. In lymphoid organs, we investigated germinal centres and various immune cell types in-depth. Human PBMCs collected prior to death were analyzed by flowcytometry. Experimental mouse models were set up to allow analysis of the immune response against RABV infection.
A severe depletion of lymphoid organs was observed in post mortem analysis of human specimen, animals and experimentally infected mice. Analysis of specific circulating human B and T cell subsets demonstrated that the humoral immune response was decreased upon RABV infection, when compared to control groups.
In conclusion, RABV infection leads to lymphoid depletion and an ineffective systemic humoral response. We hypothesize that specific innate cell types are affected by RABV early upon infection and supress the adaptive immune response.

## T-lymphocyte homing signatures in human Lassa fever

Julia Port[1], David Wozniak[2], Elisa Pallasch[1], Yemisi Ighodalo[3], Stephan Günther[1], Lisa Oestereich[1], César Muñoz-Fontela[1]

[1]Bernhard-Nocht-Institute for Tropical Medicine
[2]Robert-Koch-Institute
[3]Irrua Specialist Teaching Hospital, Nigeria

*Keywords: Lassa fever, T cells, immunity, homing*

Lassa virus (LASV) is endemic in several West African countries and causes around 300,000 symptomatic cases of Lassa fever (LF) annually. Previous work has highlighted the role of T cell-mediated immunity in LF survival. However, the relationship between T cells and LF pathophysiology is poorly understood. To gain insight into the role of T cells during LF we used transcriptomics and flow cytometry to characterize acute-phase peripheral blood samples of patients from the 2017/2018 LF epidemic in Nigeria.
Our findings indicate distinct signatures in humans that define LF survival and severity. Fatal LF was characterized by CD4 T cell recruitment to the intestinal mucosa, suggesting that, high levels of inflammation and virus replication in the gastrointestinal tract, is a predictor of poor outcome. Moreover, within survivors, severe cases showed significant recruitment of CD8 T cells to mucosae and skin. These findings were also recapitulated in a chimeric mouse model infected with LASV via different routes. In agreement with these findings, epidemiological data collected during the 2017/2018 LF epidemic in Nigeria suggests that consumption of contaminated food is the main risk associated to LF transmission. We speculate that evaluation of T cell homing can provide insight into LF kinetics and pathogenesis. Placing this data into the context of the epidemiological background could help to identify exposure risks, which has implications for public health policies.

## Novel therapeutic approach to cure Lassa fever

L. Oestereich[1,2], S. Bockholt[1,2], E. Pallasch[1,2], S. Günther[1,2]

[1] Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany
[2] German Center for Infection Research (DZIF), Partner site Hamburg, Germany

*Keywords: Lassa fever, immunmodulation, therapy, animal model*

Lassa virus (LASV) is a single-stranded RNA virus belonging to the Arenavirus family and is endemic in several regions in Western Africa. This rodent-borne pathogen can cause a severe hemorrhagic fever in humans leading up to 5000 deaths per year. Due to its epidemic potential and the current lack of specific treatments or licensed vaccines, LASV has been recently classified as a priority pathogen by the WHO.
Studies with human samples revealed that a dysregulation of homeostasis, strong inflammation and vascular dysfunction are hallmarks of Lassa fever (LF). Immunocompetent laboratory mice are resistant to LASV infection and only immunodeficient mice have shown some degree of susceptibility to infection. Since immunopathology is thought to be a key component of LASV pathogenesis, we developed a susceptible mouse model with intact adaptive, hematopoietic-driven immune response. Using transplantation of wild-type bone marrow cells into IFNAR-/- mice, we generated chimeric mice that reproduced the main features of severe Lassa fever in humans.
We used this mouse model to screen a wide range of commercially available immune modulatory drugs to treat LF and could identify two candidates that were able to reduce the disease symptoms to a remarkable degree and treated animals survived the infection. The treatment success with these two drugs represents a novel approach to treat LF, targeting the underlaying disease causing mechanisms rather that virus replication.

## *Coxiella burnetii* escapes cellular self-defense of infected NK cells

Svea Matthiesen[1], Kati Franzke[2], Rico Jahnke[1] & Michael R. Knittler[1]

[1] Institute of Immunology, Friedrich-Loeffler-Institut, Federal Research Institute of Animal Health, Greifswald - Isle of Riems, Germany
[2]Institute of Infectology, Friedrich-Loeffler-Institut, Federal Research Institute of Animal Health, Greifswald, Isle of Riems, Germany

*Keywords: Coxiella, NK cells, IFN-γ, degranulation*

Natural killer (NK) cells are critically involved in the early response against various intracellular pathogens. NK cell-mediated IFN-γ secretion essentially contributes to the protective immunity against Coxiella (C.) burnetii. In our former work, we could demonstrate for the first time that bacteria-infected NK cells functionally mature, induce cellular immunity and protect themselves via bacterial killing in secreted granules. In our present study, we found that also coxiella-infected NK cells avoid growth and development of the intracellular pathogen. Moreover, infected NK cells display maturation as well as IFN-γ secretion and release of coxiella-containing lytic granules. Most surprisingly and in contrast to other investigated bacterial pathogens, coxiella released by NK cell-degranulation largely retains its integrity and infectivity. Thus, it seems that coxiella has the ability to escape the newly discovered cellular self-defense mechanisms of infected NK cells. This provides new insights into the early steps of host-pathogen interaction, bacterial spreading and the initial immune response during C. burnetii infections.

## Session 2: Public Health

**October 16, 2019**
**17:20 – 18:50**

**Room Ballsaal**
**Chairs: Susanne Kutzora and Stefan Brockmann**

## ts-mutants of porcine influenza viruses: Pathway to live-attenuated vaccines in pigs?

Graaf A[1], Henritzi D[1], Deutskens F[2], Petrov A[2], Petric P[3], Schwemmle M[3], Beer M[1], Harder T[1]

[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald - Isle of Riems, Germany
[2]IDT Biologika GmbH & Co KG, Greifswald - Isle of Riems, Germany
[3]Institute for Virology, University Medical Center Freiburg, Germany

*Keywords: swine influenza A virus, temperature-sensitive mutants, live-attenuated influenza vaccines*

Wide spread occurrence and pathogenic potential of swine influenza A virus (swIAV) associated with respiratory diseases, cause considerable economic losses in pig production. Over the past decades in Europe, four pathogenic lineages (H1N1av, H1N2hu, H3N2, H1N1pdm/2009) emerged, some of them reassortant viruses between avian and human influenza viruses. New options to prevent respiratory symptoms in pigs by developing live vaccine candidates against infections with these four lineages are being explored. In this study, the focus is on the classical vaccine concept of temperature-sensitive (ts) live-attenuated influenza vaccines (ts-LAIV), which has yet to be evaluated in pigs. Recent field isolates of each of the four lineages have been cold-adapted (ca) through serial passaging at decreasing incubation temperature in MDCK-II and swine testicle cells. Full genome sequencing revealed multiple mutations in several gene segments compared to parental viruses. Growth kinetics established the ts-ca phenotype of the serially passaged viruses. The att phenotype will be assessed by experimental infections of mice and in the ultimate target species, pig. As swIAV have been critically involved in the development of at least the most recent human influenza pandemic virus, zoonotic potential of the vaccine virus candidates will be tested in the ferret as a human influenza model species.

## Sensibilisation of general practicioners towards imported arboviral infections in the light of established populations of *Aedes albopictus* in Baden-Württemberg 2019

Hannah Höglund-Braun[1], Christiane Wagner-Wiening[2]

[1]Department of Public Health, Heinrich-Heine University Düsseldorf
[2]Baden-Württemberg Health Authority

*Keywords: Aedes albopictus, autochthonous infections, arboviral diseases, General Practicioners, Public Health*

Since 2015 Aedes albopictus populations are spreading northward into Germany. Due to their vector competence and the adaption to colder climate Ae. albopictus bears a potential risk for transmission of arboviral infections via viremic travel returnees and thus poses a threat to Public Health in Germany. Early diagnosis of arboviral infections in regions where Ae. albopictus is established is important to reduce the risk of localized autochthonous outbreaks. To estimate the preparedness of GPs for emerging arbovirus infections a random sample of 278 GPs working in cities and counties in Baden-Wuerttemberg with proven occurrence of Ae. albopictus were interviewed by an anonymized quantitative-qualitative questionnaire. 112 participants (40%) were recruited. Of those 35% knew, that Ae. albopictus had been found in their area (47% did not; 18% unsure). 15% of all participants and 44% of those aware of the presence of Ae. albopictus had previously received information. 50% of all GPs have performed laboratory testing for Malaria (29% DNV; 6% CHIKV) and 32% have made the first diagnosis of Malaria (13% DNV; 2% CHIKV). 91% of all GPS asked wanted to receive more information on vector competent mosquitoes in Germany.
As GPs are of vital importance for monitoring the population for newly emerging diseases it is of utmost importance to increase awareness on clinical syndromes of arbovirus diseases and strengthen the preparedness by providing them with the tools and knowledge to do so.

## Tick-borne encephalitis virus isolated from natural foci causing TBE vaccine breakthroughs

Gerhard Dobler[1], Lidia Chitimia-Dobler[1], Malena Bestehorn-Willmann[2]

[1]Institut für Mikrobiologie der Bundeswehr
[2]Bereich Parasitologie, Universität Hohenheim

*Keywords: Tick-borne encephalitis, TBE vaccine, TBE vaccine breakthrough, TBE virus escape mutant*

Tick-borne encephalitis (TBE) is the most important viral encephalitis in Europe and parts of Asia. Two effective vaccines are available to prevent the tick-borne disease. However in a small part of TBE vaccinated persons show vaccine breakthroughs with manifest disease. So far it is unclear whether the reasons for TBE vaccine breakthroughs are virus immune escape mutants or they are caused by immunological failures of individual patients.
Ixodid ticks were sampled in suspected natural foci and sorted according to stage and sex. Ticks were pooled (5 adults, 10 nymphs) and crushed. The nucleic acid was extracted using a MagNA PURE extraction automate (Roche, Mannheim). TBE was detected by RT-qPCR. Extracted nucleic acid of positive pools was amplified and sequenced. Sequences were analyzed using Genious 8.1.
We identified three natural foci where TBE patients with vaccine breakthroughs got infected. TBE virus isolates from these foci were detected and were isolated and genetically analyzed. The TBE virus strains isolated did not show any common sequence pattern (especially not in the E genes) which might propose an escape mutant mechanism.
These are the first data to describe TBE virus strains which caused a TBE vaccine breakthrough. Our data show that an escape mutant mechanism probably is not the cause for TBE breakthroughs. The data imply that individual immunological deficits might be the more probable cause for TBE vaccine breakthroughs.

## Molecular epidemiology of hantavirus disease in Germany

M. Faber[1], D.H. Kruger[2], K. Stark[1], J. Hofmann[2], S. Weiss[2]

[1]Robert Koch Insitute, Berlin, Germany
[2]Institute of Virology, Charité Universitätsmedizin Berlin, Germany

*Keywords: hantavirus epidemiology, Puumala virus, Dobraba-Belgrade virus, phylogeographic analysis*

In Germany, hantavirus disease is notifiable since 2001. Two pathogenic hantavirus species, Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV, genotype Kurkino), are endemic. We analysed national hantavirus surveillance data notified to the Robert Koch Institute and hantavirus nucleotide sequences from patient samples referred to the national reference laboratory between 2001 and 2017. Matching molecular sequences with surveillance data, we conducted epidemiological and phylogeographic analyses. In total, 12,148 cases of symptomatic hantavirus infection were reported during the study period. Most cases were caused by PUUV in the south and west of Germany. Large PUUV outbreaks (2010, 2012, and 2017) showed peaks in early summer and up to 3,000 reported annual cases. DOBV infections caused few and mostly sporadic cases in autumn and winter in the north and east of Germany. A few additional (imported) Hantaan, Seoul, or DOBV-Sochi virus infections were also registered during the study period. In six cases hantavirus infection might have contributed to the death of the patients. Phylogeographic analysis show that sequence data of both, PUUV and DOBV, allow for allocation of the geographical place of infection of patients. Our data argues against an expansion of PUUV endemic areas in Germany. Since the epidemiology of PUUV and DOBV infections differs in several aspects, findings from studies on human PUUV infections might not be easily transferrable to DOBV infections.

## Prevention of zoonoses by using traditional and social media for health communication by local public health authorities (LPHA) in Germany

S.Kleele[1], C.Princk[2], M.Müller[1], M.Mylius[2], J.Dreesman[2], K.Dressel[1]

[1]SINE-Institut, Munich, Germany
[2]Governmental Institute of Public Health of Lower Saxony, Hanover, Germany

*Keywords: public health service, risk communication, qualitative methods*

**Background:** LPHA play an important role in disease prevention by communicating health risks. Authorities in Germany mainly use traditional media to inform practitioners and the public. Selecting appropriate communication tools is essential to address heterogenic target groups effectively. The interdisciplinary project RoBoPub focuses on suitable tools and strategies in Public Health (PH) communication.
**Methods:** A participative workshop with LPHA staff and qualitative interviews with representatives from LPHA and general practitioners were conducted.
**Results:** For LPHA social media use are a possibility to reach target groups already connected in existing social networks. Besides younger audiences, non-natives can be addressed more adequately and language barriers can be reduced because translation become easier. Otherwise, spreading undirected information, which is amplifying the general information overload is problematic and bears the risk of fostering uncertainty instead of awareness.
**Conclusions:** Content, distribution and timing of the message must be adjusted to the respective target group, but also to the regional and seasonal characteristics of the disease. Social media use in certain settings by LPHA could facilitate a more effective risk communication of prevention messages towards specific target groups that are otherwise hard to reach. The challenges of social media use for LPHA health communication in Germany and options, how social media could be suitably integrated in PH communication will be presented.

## Impact of hygiene- and management measures on the ESBL- and AmpC-colonization of broiler chickens

C. Robé[1], K. Daehre[1], R. Merle[2], S. Fiedler[3], C. Ewers[3], S. Guenther[4], U. Roesler[1]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany
[2]Institute for Veterinary Epidemiology and Biostatistics, Freie Universität Berlin, Berlin, Germany [3]Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University, Giessen, Germany; [4]Pharmaceutical Biology, Universität Greifswald, Greifswald, Germany

*Keywords: ESBL, broiler chickens, colonization, Escherichia coli, seeder bird model*

Background: The colonization of broilers with ESBL- and AmpC- producing Enterobacteriaceae is well-known, but only limited data on intervention strategies to reduce the colonization of broilers is available. To investigate potential measures, a recently established colonization model was used.
Material and methods: Groups of 90 broilers were housed in conventionally, alternating one measure each. Tested parameters included (1) acidification of water, (2) usage of an alternative breed, (3) reduction of stocking density to 25 kg/$m^2$, (4) tripled amount of litter, (5) application of one defined non-pathogenic Competitive Exclusion (CE-) strain and (6) of a non-defined CE-culture. One fifth of the broilers were orally co-infected on their third day of life (seeder) with $10^2$ cfu of one ESBL- and one AmpC- producing E. coli strain. Colonization of all seeder and 28 non- infected broilers (sentinel) was proven by cloacal swabs during each trial and a final necropsy.
Results and conclusion: Statistical analysis reveals a strain-dependent reduction of colonization for the ESBL- producing E. coli strain concerning the reduction of stocking density and the application of a CE-strain. Reduction of both strains was achieved using the CE-culture. In contrast, water acidification seems to increase the colonization with both bacterial strains. Consequently, the effects of the tested measures on the ESBL- and AmpC- colonization are different, with the CE as a meaningful approach for reduction.

## Session 3: New and Re-Emerging Zoonotic Diseases

**October 17, 2019**
**09:00 – 10:30**

**Room: Ballsaal**
**Chairs: Asisa Volz and Christian Drosten**

## Genetic diversity of Treponema pallidum subsp. pertenue infecting wild non-human primates in Taï National Park (TNP), Cote d'Ivoire

Benjamin Mubemba, Jan Gogarten , Ariane Düx , Markus Ulrich, Kamilla Pléh, Fabian Leendertz, Sebastien Calvignac-Spencer

Epidemiology of highly pathogenic micro-organisms, Robert Koch-Institut, Germany

*Keywords: Non-human primates, yaws, genetic diversity*

Individuals in several populations of wild non-human primates (NHP) in sub-Saharan Africa are infected with Treponema pallidum subsp. pertenue (TPE), a pathogen causing yaws disease in humans. In humans, yaws is characterized by lesions of the extremities and face. In contrast, Treponema pallidum subsp. pallidum, which causes venereal syphilis in humans, has not been observed in NHPs. We describe a combination of yaws- and syphilis-like symptoms in sooty mangabeys (Cercocebus atys atys) in Taï National Park (TNP), Cote d'Ivoire. Mangabeys presented with both yaws-like lesions on the face, body, and extremities and syphilis-like anogenital lesions. We sampled lesioned animals and collected primate bones from two additional NHPs species. We generated full Treponema pallidum genomes from biopsies and swabs and partial genomes from bones using in-solution capture coupled with next generation sequencing. Phylogenomic analysis revealed that syphilis-like lesions and yaws-like lesions were caused by TPE. Simian TPE isolates did not form monophyletic clades based on the host species or the types of symptoms caused by an isolate but rather clustered according to geographical origin. We discuss these findings in relation to other human and NHPs isolates and how this might inform ongoing yaws eradication efforts.

## Development and characterization of small animal models for variegated squirrel bornavirus 1 (VSBV-1)

Kore Schlottau[1], Daniel Nobach[2], Christiane Herden[2,3], Martin Beer[1] and Donata Hoffmann[1]

[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[2]Justus-Liebig-Universität, Institut für Veterinär-Pathologie, Gießen, Germany
[3]Center of Mind, Brain and Behavior, Justus-Liebig-University Gießen, Gießen, Germany

*Keywords: animal model, bornavirus, rodents, encephalitis*

Until the variegated squirrel bornavirus 1 (VSBV-1) was discovered in 2015, it was assumed that no member of the family Bornaviridae could infect humans. VSBV-1 was detected in four squirrel breeders and one animal caretaker, of whom four died due to a severe encephalitis. The aim of this study was to test the susceptibility of different rodent models for VSBV-1 and to characterize the course of disease. VSBV-1 infection studies were done with rats, bank voles and mice. Neonatal and adult animals were infected with a VSBV-1 cell culture isolate by different infection routes (intracerebral, intranasal or subcutaneous) and monitored for signs of disease and virus shedding. After three or five months, animals were dissected and tissue material tested by RT-qPCR and immunohistochemistry.
None of the animals showed any clinical disease and no virus shedding was observed. Despite that, some intracerebrally infected rats and bank voles were susceptible to infection as shown by high viral loads in their brains. Gross pathology showed no changes whereas histology revealed a non purulent encephalomyelitis with perivascular infiltrates in some of the neonatally infected rats.
So far, none of the tested rodents fulfilled all criteria to serve as an infection model for VSBV-1 reflecting either the reservoir host assumed to be squirrels or a dead-end host represented by humans. Further research will focus on the development of suitable animal models for future countermeasure progress.

## Fatal human variegated squirrel bornavirus 1 encephalitis is characterized by immunopathological changes

Dennis Tappe, Jonas-Schmidt-Chanasit, Jessica Rauch, Christiane Herden

*Keywords: CD4 cells; CD8 cells; microglia response; astrocyte expansion; apoptosis*

The variegated squirrel bornavirus (VSBV-1) is an emerging zoonotic virus that causes fatal panencephalitis in humans after contact to exotic squirrels. We analyzed the brain lesions and the cerebrospinal fluid responses in all four known human cases. Strong microglial response and bizarre astroglial expansion was present. Areas of malacia contained neutrophils and foamy microglia and macrophages. Inflammatory infiltrates in areas positive for VSBV-1 RNA and antigen consisted of CD4+ and CD8+ T cells, with perivascular B cell accumulation. Immunopathology during infection showed cleavage of caspase 3 in brain cells adjacent to CD8+ cells and widespread p53 expression, hallmarks of apoptosis. Cerebrospinal fluid analyses over time demonstrated increasing protein concentrations and cell counts, paralleled by pathological lactate elevations in all patients. The most severe cerebrospinal fluid and histological changes were seen in the patient with the highest viral load, shortest duration of disease, and most medical preconditions.

## Isolation and characterization of Puumala orthohantavirus from Germany

Florian Binder[1], Marion Saathoff[2], Jona Freise[2], Rainer G. Ulrich[1]

[1] Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany
[2] Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittel-sicherheit, Oldenburg, Germany

*Keywords: virus isolation, cell culture, Puumala virus, bank vole*

Puumala orthohantavirus (PUUV) is the aetiological agent of nephropathia epidemica (NE), a mild to moderate form of haemorrhagic fever with renal syndrome, which occurs in Fennoscandia, Central and Western Europe and Russia. In Germany, NE-like disease is notifiable since 2001 and the number of hantavirus cases showed high oscillations between the years. This is mainly caused by beach mast-driven bank vole population dynamics. However, German PUUV strains have never been isolated in cell culture and characterized. In this study, virus isolation attempts were carried out in the field, inoculating naïve bank vole kidney-derived and Vero E6 cell lines with freshly homogenized lung tissue. As an outcome, three virus strains were isolated from bank voles (Myodes glareolus) trapped in forests close to Osnabrück in Lower Saxony in 2019. The bank vole-derived strains were identified by PUUV specific real-time and conventional RT-PCR assays and sequence analysis of the S segment. Phylogenetic analysis of the novel PUUV sequences indicated a clustering within the central European clade of PUUV together with other local PUUV sequences. Growth kinetics were determined in both, VeroE6 and MGN-2-R cells, and virus sequence evolution in both cell lines is currently being investigated. In conclusion, a novel protocol for PUUV isolation from bank vole lung tissue was successfully established.

## In vivo evaluation of a MVA based Zika Virus (ZikV) antigen library in a mouse model for ZikV disease

Jan Schwarz, Dominik Forster, Asisa Volz, Gerd Sutter

Institute for Infectious diseases and zoonoses, Chair for Virology, LMU Munich, Germany

*Keywords: Zika Virus, vaccination, Emergency vaccination*

Emerging zoonotic pathogens necessitate the rapid development of new vaccines that confer protection in case of an outbreak scenario. This has been imposingly demonstrated by the sudden emergence of Zika Virus ZikV) in South America in 2015/2016. An innovative approach to rapidly test candidate vaccines is the comparative expression of different ZikV antigens that can be evaluated in vivo for their protective capacity. For this, we established a "ZikV-ag-library" using the MVA as highly versatile expression system. In the next step these ZIKV proteins will be comparatively tested in vivo for the induction of protection against lethal challenge infection. Interestingly, we observed differences in the outcome of ZikV clinical disease that could be associated with specific immune responses.
Whereas some antigens fully protected mice against ZikV infection, we were able to identify significant differences in clinical outcome and immunogenicity between some other recombinant MVA constructs. With these findings we want to contribute to the development of improved candidate vaccines.

## New "omics"-insights into the genome evolution and virulence potential of the emerging group of marine Brucella species

Dirk Hofreuter[1], Holger Brendebach[1], Sandro Andreotti[2], Kerstin Neubert[2], Boyke Bunk[3], Matthias Flor[1], Josephine Grützke[1], Cathrin Spröer[3], David Fretin[4], Jörg Overmann[3] and Sascha Al Dahouk[1]

[1] German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany
[2] Freie Universität Berlin, Department of Mathematics and Computer Science, Berlin, Germany
[3] Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
[4] Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre, Brussels, Belgium

*Keywords: emerging Brucella species, NGS, pathogenomics, host tropism, metabolic phenotyping*

Brucellosis is a zoonotic disease of livestock, wildlife and humans worldwide. In terrestrial mammals, Brucella abortus, B. melitensis and B. suis are most prevalent. However, brucellae have also been isolated from marine mammals with phenotypic properties distinct from the classical species. Consequently, the novel species B. ceti and B. pinnipedialis, named after their host preferences, were defined.

Human infections caused by B. ceti strains of the sequence type ST27 have been reported, suggesting a zoonotic potential of marine Brucella species. To improve our knowledge about the biology and host specificity of marine Brucella species, we applied extensive Illumina® third-generation DNA sequencing of 65 B. ceti and 120 B. pinnipedialis isolates from dolphins, whales, and seals. We completed the genomic information by PacBio long-read DNA sequencing of seven selected strains to get a better understanding of how repetitive transposable elements drive the structure and evolution of the genomes.

Additional metabolic profiling of the Brucella strains by Micronaut™ phenotyping allowed us to identify various distinct substrate utilization patterns in the two marine species that might reflect adaptations to their specific hosts.

Finally, comprehensive genome comparisons revealed that in addition to genomic islands, extensive genetic microdiversity seems to be the driving force behind the diverse sets of active virulence factors in the distinct groups of marine brucellae.

## Session 4: Diagnostics and NGS

**October 17, 2019**
**09:00 – 10:30**

**Room Zehlendorf**
**Chairs: Victor Corman and Martin Beer**

## Fast differentiation of Enterobacteriaceae using Raman Spectroscopy

Amir Nakar[1,2,3], Karina Weber[1,2,3], Sascha D.Braun[1,3], Ralf Ehricht[1,2,3], Petra Rösch[2,3] and Jürgen Popp[1,2,3]

[1]Leibniz Institute of Photonic Technology Jena - Member of the research alliance "Leibniz Health Technologies", Albert-Einstein-Str. 9, 07745 Jena, Germany
[2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena, Jena, Germany
[3]Research Campus Infectognostics, Germany

*Keywords: Raman, Spectroscopy, Enterobacteriaceae, Carbapenem resistance, Chemometrics*

Enterobacteriaceae is a family of bacteria, many of whom are zoonotic pathogens, such as Escherichia, Shigella and Klebsiella species. Identifying these bacteria is critical for tracking outbreaks of zoonosis. However, Enterobacteriaceae share some characteristics that make them difficult to differentiate (for example, Shigella and Escherichia). By measuring the Raman spectrum of bacterial cells, it is possible to rapidly collect molecular fingerprints. These can be used to identify different bacteria. In this study, Raman spectroscopy was applied for fast differentiation of strains of Enterobacteriaceae. We used spectra collected from single bacterial cells. In an attempt to create a large database, we collected different strains from different sources, including both sensitive and multi-drug resistant strains and used molecular typing methods prior to Raman spectroscopy. With this database, we trained a chemometric model to identify the strains based on their spectra. We could identify bacteria at the species and genus level. Furthermore, we trained a model to identify strains that carry antibiotic resistance to Carbapenem. These early results indicate that this method may be applicable, however in order to improve accuracy the database needs to be extended. We hope to improve identification of Enterobacteriaceae and thus advance public health.

We thank the Federal Ministry of Education and Research (BMBF) for funding this research as part of project CarbaTech (01EI 1701).

## Influence of infection route on neuropathology of tick-borne encephalitis virus in a murine infection model

Mathias Boelke[1,4], Christina Puff[2], Fanny Naccache[1,4], Frederic Gusmag[1,4], Katrin Liebig[1,4], Sonja T. Jesse[4], Andreas Beineke[2], Gerhard Dobler[3], Ab D.M.E. Osterhaus[4], Wolfgang Baumgärtner[2], Claudia Schulz[4] and Stefanie C. Becker[1,4]

[1]Institute for Parasitology, University of Veterinary Medicine, Buenteweg 17, 30559 Hanover, Germany
[2]Department of Pathology, University of Veterinary Medicine, Buenteweg 17, 30559 Hanover, Germany
[3]Institute of Microbiology of the Bundeswehr, Neuherbergstraße 11, 80937 Munich, Germany
[4]Research Center for Emerging Infections and Zoonoses, Buenteweg 17, 30559 Hanover, Germany

*Keywords: Tick-borne encephalitis, mouse infection model, tick bite, per os application*

Tick-borne encephalitis virus (TBEV) is an important vector-borne zoonotic flavivirus with a high impact on public health. Mechanisms of TBEV- invasion and damage in the central nervous system are scarcely understood. Potential determinates are genetic factors within the host or virus strain and might be influenced by the route of transmission. Regarding the question of disease severity, it is of special interest that TBEV may not only be transmitted by a bite of a TBEV-infected tick, but also directly between vertebrate hosts via consumption of unpasteurized milk or dairy products of infected animals. While the subcutaneous route is a well-established method to test the neuroinvasivness of TBEV strains, few studies have been conducted using oral application or tick bite infection in a murine infection model. Therefore, we compared different natural TBEV-infection routes in a murine infection model using C57BL/6 mice. The mice were experimentally infected with the TBEV strain Neudoerfl either by tick bite, subcutaneous injection or by oral application. Virus replication kinetics in various organs and disease development were compared to find potential associations of infection route-specific influences on the neuropathogenesis. Furthermore, virus sequences obtained from mouse samples collected during the different trials will be analyzed for potential infection route-specific adaptations using Next Generation Sequencing.

**Acknowledgement:** This project is funded by the Niedersachsen Research Network on Neuroinfectiology of the Ministry of Science and Culture of Lower Saxony (N-RENNT), the Volkswagen Foundation and Tick-Borne Encephalitis in Germany (TBENAGER). We would like to thank the Bundeswehr for financial support.

## Rabies virus in Namibian Kudus – Exploring the Genetic Diversity of Lyssaviruses

Sten Calvelage[1], Conrad Freuling[2], Thomas Müller[2], Martin Beer[1], Dirk Höper[1]

[1]Friedrich-Loeffler-Institute - Institute of Diagnostic Virology
[2]Friedrich-Loeffler-Institute - Institute of Molecular Virology and Cell Biology

Keywords: Rabies Lyssavirus, Kudu, Variant Analysis, Deep Sequencing

While rabies is one of the oldest known zoonotic diseases, knowledge of important key mechanism like adaption processes of rabies lyssavirus (RABV) to new host species is still incomplete. Rabies in the Greater Kudu (Tragelaphus strepsiceros) in Namibia is unique and found in such magnitude as it has not been reported elsewhere in southern Africa. While experimental studies could demonstrate the possibility of horizontal RABV transmission to contact animals, the rapid spread of the disease in Kudus over large territories remains elusive. To elucidate the potential genetic diversity of the RABV genome in Kudus that may explain species adaption, a sample set of salivary gland and brain materials from infected Kudus was selected and analyzed by a powerful next generation sequencing workflow. The results revealed the consistency of the viral consensus sequences in the two different organs of an infected individual. However, several substitutions distributed across the whole virus genome, except the N gene, with frequencies ranging from 1.6 % up to 39 % were identified, most of them located in coding regions of different genes. Among these substitutions, a high number of nonsynonymous mutations exist, leading to various amino acid exchanges within most of the viral proteins. The frequencies of the observed mutations changed between brain and salivary gland material, demonstrating the genetic variability of RABV in the Greater Kudu underneath the consensus level.

## Concordance between genomic typing methods can simplify data exchange for surveillance and control of infectious diseases

S. Lüth[1,2], C. Deneke[1], S. Kleta[1], S. Al Dahouk[1,3]

[1]German Federal Institute for Risk Assessment
[2]Freie Universität Berlin;
[3]RWTH Aachen University

*Keywords: Genomic Epidemiology, Whole Genome Sequencing, Phylogenetic Relatedness, Core genome MLST, Single Nucleotide Polymorphism*

Where classical epidemiology has proven to be inadequate for surveillance of pathogens, molecular epidemiology, using genomic typing methods, can add value. However, procedures for analysis of whole genome sequencing (WGS) data are diverse and still hardly standardised.
We used WGS data of 511 Listeria monocytogenes (Lm) isolates from 50 different MLST clonal complexes of the strain collection of the National Reference Laboratory for Lm as a test dataset to compare procedures for WGS data analysis and to evaluate concordance of results. Different bioinformatics approaches (e.g. BioNumerics, command-line based in-house pipelines) for gene-by-gene (core genome MLST) and single nucleotide polymorphism (SNP) analysis were tested. The extent of detected differences among isolates varied considerably between approaches whereas the degree of phylogenetic relatedness between isolates was largely comparable. While a general reference was well suited for an overview of the population structure, a closely related reference genome, draft just like closed, increased resolution of SNP analysis by up to threefold.
To create the basis for WGS as a routine technique in molecular epidemiology, either free and rapid exchange of WGS data has to be guaranteed or comparability of typing results yielded by different bioinformatics approaches needs to be harmonised. Our results revealed a high concordance between common analysis tools which opens up a new perspective independent of standardisation.

## Genome-Wide Identification of Genomic Determinants for Host Restriction in *Campylobacter jejuni*

Lennard Epping[1], Rosario M. Piro[2], Marie-Theres Knüver[3], Maria Borowiak[4], Charlotte Huber[5], Andrea Thürmer[6], Burkhard Malorny[4], Kerstin Stingl[3], Angelika Fruth[7], Lothar H. Wieler[6], Torsten Semmler[1]

[1]Microbial Genomics, Robert Koch Institute, Berlin, Germany
[2]Department of Mathematics and Computer Science, Freie Universität, Berlin, Germany; Institute of Medical Genetics and Human Genetics, Charité Universitätsmedizin, Berlin, Germany
[3]National Reference Laboratory for Campylobacter, Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[4]Study Centre for Genome Sequencing and Analysis, Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[5]Advanced Light and Electron Microscopy, Robert Koch Institute, Berlin, Germany
[6]Methodology and Research Infrastructure, Robert Koch Institute, Berlin, Germany
[7]National Reference Centre for Salmonella and other Bacterial Enterics, Robert Koch Institute, Wernigerode, Germany

*Keywords: Campylobacter; Whole Genome Sequencing; Genome-Wide Association Study; Host Specificity*

The zoonotic pathogen Campylobacter jejuni is the leading cause of bacterial food-borne illness in humans in industrialized countries. The contamination of chicken meat or raw milk products are the main transmission ways to human. Multi locus sequence typing (MLST) based on C. jejuni genomic data from different sources has shown that certain sequence types (STs) are associated with specific hosts or a host-generalism lifestyle. However, the survival mechanisms of C. jejuni to adapt to different gut environments have not been completely understood yet.
To generate more in-depth knowledge about these mechanisms, we randomly selected and whole genome sequenced 330 C. jejuni strains from different hosts across Germany. We investigated the host-specificity by a k-mer based Genome-Wide Association approach. To increase the statistical power of the study, 166 genomes from other published international studies were included.
In this study, we discovered a strong host association within the core genome as well as the accessory genome. Furthermore, genetic elements that play important roles in mobility, energy metabolism and DNA replication processes were discovered.
The host-adaptation in a wide range of cellular functions in the whole pan-genome of C. jejuni indicates that the adaptation towards a specific host niche is most likely a long evolutionary and multifactorial process rather than a spontaneous evolution due to selection pressure.

## Identification of host-associated genomic determinants in *Escherichia coli*

Sumeet Kumar Tiwari[1], Boas Van der Putten[2], Trung Nguyen V.[3], Martin Bootsma[4], Roberto La Ragione[5], Sebastien Matamoros[6], Hoa Ngo T.[7], Christian Berens[8], Joy Leng[9], Julio Álvarez[10], Dominique Jolivet[2], Marta Ferrandis Vila[11], Jenny Ritchie[12], Angelika Fruth[13], Stefan Schwarz[14], Lucas Domínguez[15], María Ugarte-Ruiz[10], Astrid Bethe[16], Lothar H. Wieler[17], Christian Menge[8], Amanda Fivian-Hughes[18], Constance Schultsz[2], Torsten Semmler[19]

[1]Robert Koch Institute, Microbial Genomics, Berlin, Germany
[2]Amsterdam UMC, Department of Global Health, Amsterdam, Netherlands
[3]Oxford University Clinical Research Unit, Vietnam
[4]UMC Utrecht, Utrecht, Netherlands
[5]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, UK.
[6]Amsterdam UMC, Department of Medical Microbiology, Amsterdam, Netherlands
[7]Nuffield Department of Medicine, University of Oxford, Oxford OX3 7BN, UK
[8]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany
[9]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, UK
[10]VISAVET Health Surveillance Center, Universidad Complutense, Madrid, Spain
[11]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany
[12]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, UK
[13]Robert Koch Institute, Enteropathogenic Bacteria and Legionella, Wernigerode, Germany
[14]Freie Universität Berlin, Berlin, Germany
[15]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain
[16]Freie Universität Berlin, Berlin, Germany
[17]Robert Koch Institute, Berlin, Germany
[18]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, UK
[19]Robert Koch Institute, Microbial Genomics, Berlin, Germany

*Keywords: Host Adaptation, E. coli, Population Genomics, GWAS*

Escherichia coli, a multi-host and commensal bacterium, can act as reservoir and disseminator of antimicrobial resistance (AMR) genes in and between humans, and animals. However, to date there is a paucity of data available relating to how certain lineages of E. coli are adapted to a specific host or have lost such a specialization. Which genomic determinants contribute to the host adaptation and have an impact on a narrow or broad host range is also unclear. This study aimed to identify the genome-wide host adaptive genes within E. coli and their effect on the transmission and spread of AMR and bacterial fitness.
A panel of 1,199 E. coli isolates (2003-2018) from cattle, chickens, humans and pigs and from Germany, Spain, UK and Vietnam were randomly selected for whole-genome sequencing and phenotypic characterization. Host-association of enriched genes in these E. coli was evaluated by genome-wide association study (GWAS) approaches. The core and accessory gene clustering patterns show lineages adapted to specific hosts. We observed adaptation of ST131 and ST117 to human and chicken hosts, respectively. Strains from diseased hosts, while present across the entire bacterial population, appeared enriched in phylogenetic lineages D, F and B2. Neither the virulence genes, nor the AMR gene profiles were associated with either host or phylogenetic lineages. The GWAS analysis showed a statistically significant association of certain genes or allelic gene variants to specific hosts.

## Session 5: Epidemiology and Ecology of zoonotic infections I

**October 17, 2019**
**09:00 – 10:30**

**Room: Steglitz**
**Chairs: Jonas Schmidt-Chanasit and Ard Nijhof**

## Are ESBL- producing *E. coli* spread in the environment by manure application, soil cultivation and wind erosion?

Paul Siller[1], Katrin Daehre[1,2], Uwe Roesler[1]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany
[2]Federal Office of Consumer Protection and Food Safety, Berlin, Germany

*Keywords: ESBL, E. coli, Environment, Manure, Wind erosion*

**Background and objectives:** Broiler and pig manure frequently contains ESBL-producing E. coli and is used to fertilize arable land. To estimate the risk of an airborne spread of these resistant bacteria from organic fertilizers to the environment, we performed field, wind channel and short-term manure storage trials.
**Materials and Methods:** In field trials, we took air and soil samples while and after ESBL-containing fertilizer application and agricultural cultivation. In wind channel trials, an ESBL-positive soil-manure mix was exposed to different wind speeds to simulate wind erosion. In manure storage trials in summer and winter, the number of ESBL- E. coli was monitored in a chicken manure pile. All samples were qualitatively and quantitatively analysed. Selected isolates were analysed by NGS.
**Results:** In the field and wind channel experiments, ESBL- E. coli were not detected in the air samples. In one field trial with pig slurry, ESBL- E. coli were detected in soil samples after 33 days. In the manure storage trials we saw an immediate and rapid decline in ESBL- E. coli numbers.
**Conclusion:** The amount of ESBL- E. coli in organic fertilizers drops immediately after leaving the stables. When incorporated to soil, they can survive for at least one month, but ESBL- E. coli seem to have a low survival rate when aerosolized. Storing manure before land application is effective to reduce bacterial counts. Considering all results, an airborne environmental spread seems unlikely.

## Cross-Protection of Dengue Virus Infection against Congenital Zika Syndrome, Northeastern Brazil

Celia Pedroso[1], Carlo Fischer[2], Marie Feldmann[3], Eduardo Martins Netto[1], Beate M. Kümmerer[4], Jan Felix Drexler[5]

[1]Universidade Federal de Bahia, Salvador, Brazil
[2]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin Humbolt-Universität zu Berlin and Berlin Institute of Health, Institute of Virology, Berlin, Germany
[3]University of Bonn Medical Centre, Bonn, Germany
[4]University of Bonn Medical Centre, Bonn, Germany
[5]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin Humbolt-Universität zu Berlin and Berlin Institute of Health, Institute of Virology, Berlin, Germany

*Keywords: Antibody-dependent enhancement, Zika virus, cross-protection, Dengue virus, northeastern Brazil*

The Zika virus (ZIKV) outbreak in Latin America has a major impact on maternal and child health due to ZIKV-associated congenital malformations (CZS). Data from in vitro studies suggest that dengue virus (DENV)-mediated immune enhancement may affect CZS formation. Our goal was to examine whether preexisting DENV immunity did affect CZS formation within a case-control framework in northeastern Brazil.

We analyzed historical DENV genomic data to compare genomic signatures from different Brazilian regions. Moreover, we performed serotype-specific plaque-reduction neutralization tests (PRNT90) for DENV and ZIKV for 29 ZIKV-seropositive mothers of CZS cases and 108 ZIKV-seropositive controls from northeastern Brazil.

No unique DENV genomic signature was evident for northeastern Brazil, refuting differential anti-DENV immunity to affect CZS incidence. DENV seroprevalence was significantly higher in controls than in cases ($p=0.0003$). The median number of neutralized DENV serotypes was two for cases and four for controls ($p<0.0004$). Serotype-specific DENV neutralization was neither correlated with ZIKV titers, nor with heterologous DENV titers, suggesting robustness of the serological data.

Our study strongly suggests a complex interaction between ZIKV and DENV immunity and a protective effect of preexisting multitypic DENV immunity of the mother on CZS development in the fetus during the Zika virus outbreak in northeastern Brazil.

## Tropical rainforest flies carrying pathogens form stable associations with social non-human primates

Jan Frederik Gogarten[1], Ariane Düx[1], Benjamin Mubemba[1], Kamilla Pléh[1], Constanze Hoffmann[1], Alexander Mielke[2], Jonathan Müller-Tiburtius[1], Andreas Sachse[1], Roman Wittig[2], Sébastien Calvignac-Spencer[1], Fabian Leendertz[1]

[1]Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institute
[2]Primatology Department, Max Planck Institute for Evolutionary Anthropology

*Keywords: Anthrax; Yaws; Vector; Disease risk; Sociality*

Living in groups provides benefits but incurs costs such as attracting disease vectors. For example, synanthropic flies associate with human settlements, and higher fly densities increase pathogen transmission. We investigated whether such associations also exist in highly mobile non-human primate groups (NHP). We studied flies in a group of wild sooty mangabeys (Cercocebus atys atys) and three communities of wild chimpanzees (Pan troglodytes verus) in Taï National Park, Côte d'Ivoire. We observed higher fly densities within mangabey and chimpanzee groups. Using a mark-recapture experiment, we showed that flies stayed with the mangabey group for up to 12 days and for up to 1.3 km. We tested mangabey associated flies for pathogens infecting mangabeys in this ecosystem, Bacillus cereus biovar anthracis (Bcbva), causing sylvatic anthrax, and Treponema pallidum pertenue, causing yaws. Flies contained treponemal (6/103) and Bcbva (7/103) DNA. We cultured Bcbva from all PCR-positive flies, confirming bacterial viability. Whole genome sequences of Bcbva isolates revealed a diversity of Bcbva, likely derived from several sources. We conclude that flies actively track mangabeys and carry infectious bacterial pathogens. Determining whether NHP-associated flies move into surrounding human populations is an important area of future research and we present a preliminary analysis of the mammalian and pathogen DNA found in 45 flies captured in a village next to the park.

## Does cold winter really limit the dengue vector *Aedes aegypti* in Europe? – A meta analysis and experimental evaluation of the cold tolerance *of Ae. aegypti* versus *Ae. albopictus* eggs

Isabelle Kramer[1], Christian Scherer[1], Aljoscha Kreß[1], Meghnath Dhimal[2], David A. Groneberg[1], Bodo Ahrens[3], Ulrich Kuch[1], Parbati Phuyal[1], Doris Klingelhöfer[1], Ruth Müller[4]

[1]Institute of Occupational, Social and Environmental Medicine, Frankfurt am Main- Germany
[2]National Health Research Council, Kathmandu, Nepal
[3]Institute for Atmospheric and Environmental Science, Goethe University Frankfurt, Frankfurt, Germany
[4]Institute of Tropical Medicine, Antwerp, Belgium

*Keywords: Aedes aegypti, Aedes albopictus, cold tolerance, distribution limits, winter survival*

*Aedes aegypti* is a feared vector for several arboviruses including dengue virus. Nowadays, the species seems to be restricted to their subtropical/tropical habitats and has difficulties to re-establish permanent populations in Europe that can overwinter. The aim of this study was a systematic analysis of cold tolerance of eggs of *Ae. aegypti* and a comparison with well-established *Ae. albopictus* in Europe by systematically studying the literature and adding experimental data. Zero and subzero temperatures are rarely tested in *Ae. aegypti* in comparison to *Ae. albopictus.* Those studies imply a certain cold tolerance in *Ae. aegypti* eggs, which we experimentally tested in more detail. We conducted cold tolerance experiments with eggs of non-diapausing subtropical populations of both species. In brief, survival (hatching in %) of 100 non-diapausing eggs of both species and emergence of adults were tested after exposure to low and sub-zero temperature for different exposure durations (3°C, 0°C and - 2°C: ≤9 days; - 6°C: ≤2 days). The *Ae. aegypti* eggs can survive low and sub-zero temperatures similarly to *Ae. albopictus*. Thus, cold winter temperature may not limit the re-establishment of the dengue vector *Ae. aegypti* in Europe. One may suspect, that *Ae. aegypti* is likely to establish in Europe again, especially with regard to climate warming.

# West Nile virus in Germany: vector competence of native *Culex pipiens* and *Aedes albopictus*

C. M. Holicki[1], U. Ziegler[1], H. Kampen[2], D. Werner[3], C. Silaghi[2], M. H. Groschup[1] and A. Vasić[2]

[1] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany
[2] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Infectology, Greifswald-Insel Riems, Germany
[3] Institute of Land Use Systems, Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany

*Keywords: West Nile virus, Culex pipiens, Aedes albopictus, vector competent*

Being a question of time, West Nile virus (WNV, Flavivirus, Flaviviridae) eventually emerged in Germany, with the first antigen detection in September 2018 in a great grey owl in the federal state of Saxony-Anhalt. Once established, WNV, like many arthropod-borne viruses, poses a continuous threat to wild and captive animal species as well as to human health. The virus is maintained in an enzootic cycle between ornithophilic mosquitoes as vectors and birds as amplifying hosts. Humans and horses are considered dead-end hosts, where an infection generally progresses asymptomatically and only in rare cases leads to neuroinvasive disease.
Multiple virus genome detections, obtained from the north to the south of Germany, raise the question of how WNV spreads throughout Germany and which vectors possibly play a key role in its transmission. Infection experiments were performed with three native mosquito colonies (Culex pipiens biotype pipiens, Culex pipiens biotype molestus, Aedes albopictus) and the WNV lineage 2 strain isolated from the first infected owl in Germany. The mosquitoes were fed with a virus-spiked blood meal and incubated for two to three weeks at 25°C, representing average summer temperatures in Germany. To assess the vector competence of a colony, mosquito saliva, bodies and extremities were tested for the presence of viable virus or viral RNA. All tested species were susceptible to a WNV infection, with individual specimens transmitting virus in their saliva

## Evaluation of the vector competence of ticks for *Coxiella burnetii* using an artificial feeding system

Sophia Körner[1,2], Gustavo R. Makert[1], Katja Mertens-Scholz[2], Klaus Henning[2], Martin Pfeffer[3], Sebastian Ulbert[1]

[1] Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany
[2] Friedrich Loeffler Institute, Jena, Germany
[3] Institute of Animal Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig, Germany

*Keywords: ticks, artificial feeding, Coxiella burnetii, Q fever*

Coxiella burnetii causes Q fever in a wide range of hosts, including humans. The main transmission route is via inhalation of contaminated aerosols, which arise during parturition of infected ruminants. Q fever is also discussed as vector-borne disease but the role of ticks in transmission of C. burnetii is unclear.
The aim of this study is to clarify the transmission of C. burnetii between tick life stages and from ticks to their hosts. Therefore, a silicone-membrane based in vitro feeding system was adapted for feeding of Ixodes ricinus with C. burnetii Nine Mile phase II inoculated blood. Feces and adult ticks were tested by real-time PCR. Furthermore, L929 cells were inoculated with feces positive for C. burnetii DNA to confirm viability of the excreted bacteria. Larvae and nymphs were fed with C. burnetii spiked blood and left for molting to assess transstadial transmission.
The artificial feeding system was established for all life stages of I. ricinus and an engorgement rate of 50% in adult ticks was achieved. First results showed a time-dependent excretion of infectious C. burnetii within feces. This demonstrates the possibility of transmission of C. burnetii by inhalation of infected tick feces. Further analysis of the reinfection process will increase our knowledge about tick-dependent Q fever transmission.

## Session 6: Epidemiology and Ecology of zoonotic infections II

**October 17, 2019**
**11:00 – 12:30**

**Room: Ballsaal**
**Chairs: Ruth Müller and Rainer Ulrich**

## Epidemiology of leptospirosis in Uganda - Starting from Scratch

Anou Dreyfus[1,2], Lordrick Alinaitwe[3], Kathryn Allan[4], Sabrina Rodriguez-Campos[5,6], Paul Torgerson[1], Jonathan W. Dyal[7], David Boulware[8], Raewynne Pearson[9], Terence Odoch[10], Charles Kajura[11], Steven Kakooza[3], Katharine Pelican[12], Dominic Travis [12], Michael Mahero[12], Christina Faust[4], Edridah M. Tukahebwa[13], Valentine Jaquier[5], Amanuel Tsegay[3], Lawrence Mugisha[10,12,14], Clovice Kankya[10]

[1] Section of Epidemiology, University of Zurich, Zurich and [2] Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland. *Presenting author.
[3] Central Diagnostic Laboratory, College of Veterinary Medicine, Animal resources and Biosecurity, Makerere University, Kampala, Uganda
[4] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Scotland
[5] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland and [6] Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway
[7] Department of Emergency Medicine, Harvard Medical School, Boston, USA
[8] Department of Medicine, University of Minnesota, Minneapolis, USA
[9] SVS Laboratories, Hamilton, New Zealand
[10] College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda
[11] Hoima District Local Government, Hoima, Uganda
[12] Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, USA
[13] Vector Control Division, Ministry of Health, Kampala Uganda
[14] Conservation & Ecosystem Health Alliance, Kampala, Uganda

Background: Prior to 5 years ago, no leptospirosis data in humans was published from Uganda. We summarize our findings of leptospirosis research at the human-animal interface in Uganda.
Methods: We conducted cross-sectional studies in Uganda of: 1) patients attending district hospitals (n=359), 2) farmed (n=275) and slaughtered (n=500) cattle, 3) rodents (n=141). Human and cattle serum was tested by microscopic agglutination to determine seroprevalence. Leptospira carrier state was tested by qPCR targeting LipL32 on kidneys and urine from slaughtered cattle, and on kidneys from rodents. We determined species of infecting Leptospira by amplification of secY gene.
Results: We identified 35% of patients seropositive, with skinning as a risk factor. Farm and slaughter cattle had an overall Leptospira seroprevalence of 19% and 28%, respectively. We identified Leptospira in 9% of cattle kidney or urine. Halal butchers and meat inspectors had a 100% risk of daily exposure to ≥1 Leptospira carrier animal. L. borgpetersenii and L. kirschneri were confirmed as infecting species. 3.5% (5/144) of rodent kidneys were Leptospira DNA positive.
Conclusions: Leptospira seroprevalence of 35% in humans suggests frequent exposure to this pathogen. Confirmation of renal infection and urinary shedding of pathogenic leptospires in cattle demonstrate that cattle act as a reservoir source. Research of leptospirosis in febrile patients and of risk factors is recommended.

## A multitaxa study on virus diversity and abundance in habitats differing in their level of disturbance reveals virus-host-specific prevalence patterns

Kyra Hermanns [1], Florian Zirkel[2], Sandra Junglen[1]

[1]Institute of Virology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]Institute of Virology, University of Bonn Medical Center, Bonn, Germany

*Keywords: Anthropogenic disturbance, virus diversity, virus prevalence, mosquitoes*

Most studies analyzing the influence of biodiversity on pathogen emergence focus on a single host-pathogen system and often observe contrary effects. It is unclear which effects may drive pathogen emergence from natural ecosystems. Here we studied virus abundance and diversity patterns in natural and disturbed ecosystems using an unbiased multitaxa approach.
Mosquitoes sampled along a disturbance gradient in Côte d'Ivoire were tested by generic RT-PCR assays established for all major arbovirus and insect-specific taxa. Phylogenetic relationships of detected viruses and viral infection rates according to habitat and host were analyzed.
We detected 34 novel and 15 known viruses pertaining to the families Flavi-, Rhabdo-, Reo-, Toga-, Mesoni- and Iflaviridae and the order Bunyavirales. Highest viral diversity was observed in pristine and intermediately disturbed habitats. The majority of the 49 viruses was found with low frequencies. However, nine viruses were found in more than 10 pools of which five increased in prevalence in disturbed habitats, congruent with the dilution effect hypothesis. These viruses were mainly associated with one specific mosquito species (Culex nebulosus), that increased in abundance from pristine (3%) to disturbed habitats (38%). Interestingly, the observed increase of virus prevalence in disturbed habitats was not caused by higher host infection rates but by higher host frequencies suggesting that population composition influences virus emergence.

## Anthroponotic transmission of *Cryptosporidium spp*. in rural sub Saharan Africa: a one health study

Daniel Eibach[1], Ralf Krumkamp[1], Simone Caccio[2], Akim Adegnika[3], John Amuasi[4], John Lusingu[5], Raphael Rakotozandrindrainy[6], Jürgen May[7]

[1] Bernhard Nocht Institute for Tropical Medicine
[2] Istituto Superiore di Sanità
[3] Centre de Recherches Médicales de Lambaréné
[4] Kumasi Centre for Collaborative Research in Tropical Medicine
[5] National Institute for Medical Research
[6] Université d'Antananarivo
[7] Bernhard nicht Institute for Tropical Medicine

*Keywords: Cryptosporidium; Contact networks, One Health, Africa*

Introduction
High prevalence and mortality from Cryptosporidiosis among children in sub-Saharan Africa has been shown in recent years, however transmission dynamics and reservoirs are yet to be investigated. This multicentre study traces back Cryptosporidium positive children to their close human and animal contacts in order to identify transmission networks.

Methods
Stool samples from children below 5 years with diarrhoea were collected at hospitals in Gabon, Ghana, Madagascar and Tanzania. Cryptosporidium positive and negative initial children were followed to the community, where stool samples from all household members, neighbouring children and animal contacts (cows, sheep, goats and dogs) were obtained. Samples were screened for Cryptosporidium spp. by PCR-RFLP analysis and subtyped (60 kDa glycoprotein gene) for C. hominis and C. parvum. Contact networks were identified and rate ratios (RR) calculated.

Results
Among 1,363 initial children 44 (20%), 47 (11%), 25 (11%) and 68 (14%) were diagnosed with Cryptosporidium spp. in Gabon, Ghana, Madagascar and Tanzania, respectively. Compared to Cryptosporidium negative initial children, positive initial children had an increased risk of having positive household members (RR = 2.5; 95%-Confidence Interval (CI): 1.5–5.2) or positive neighbouring children (RR = 2.7; 95%-CI: 1.6–4.8), but no risk of having positive animals (RR = 1.3; 95%-CI: 0.8–2.1) in their contact network.

Conclusions
Cryptosporidiosis in rural sub-Saharan Africa is characterized by clusters among human contacts, to which zoonotic transmission seems to contribute only marginally. Shared sanitation facilities or water sources may be responsible for anthroponotic neighbourhood transmission.

## Tick-borne encephalitis: First results of intensified surveillance in Bavaria and Baden-Württemberg (TBENAGER)

T. M. Kreusch[1], M. M. Böhmer[2], C. Wagner-Wiening[3], O. Wichmann[1], W. Hellenbrand[1]

[1] Robert Koch Institute, Berlin, Germany
[2] Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, Germany
[3] State Health Office Baden-Württemberg (LGA), Stuttgart, Germany

*Keywords: tick-borne encephalitis, surveillance, disease severity, risk factors, Germany*

Annually, ~337 tick-borne encephalitis (TBE) cases are notified in Germany. Surveillance data incompletely capture the true disease severity, thus we implemented an intensified surveillance for 2018–2020. We aim to fill knowledge gaps on TBE severity, risk factors for infection and reasons for non-compliance with vaccination recommendations.
We invited cases for phone interviews on acute symptoms, risk behaviours and vaccination status. In a case-control design, we additionally interviewed 2:1-matched population controls. Data were analysed using Stata 15.
In 2018, 187/429 cases (44%) participated: 64% were male, the mean age was 47 years (SD=19, range 3–86). At the interview 46% had fully recovered, while 45% reported mild and 8% moderate/severe symptoms (modified Rankin Scale=1–2; 3–4). Detailed multifactorial risk factor analyses will be presented once data are complete; notable signals include case-control differences in pet ownership (cases 49% vs. controls 36%), occupational risk (22 vs. 18%), 12-months tick count (83 vs. 70%), frequent garden use (45 vs. 36%) and garden's proximity to forest (<200m: 51 vs. 28%). The main vaccination barrier was a low perceived infection risk in both groups, although almost all participants lived in TBE risk areas, where vaccination is recommended.
The broad symptom profile characterises TBE as a severe disease in most notified cases. Better understanding risk factors and vaccination barriers will permit more targeted TBE prevention.

## Citizen science[3]: surveillance of Usutu virus activity in Germany

Renke Lühken, Daniel Cadar, Norbert Becker, Stefan Bosch, Lars Lachmann, Jonas Schmidt-Chanasit

Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

*Keywords: Usutu virus, arbovirus surveillance, early-warning system*

Usutu virus (USUV) is a mosquito-borne virus, circulating in an enzootic transmission cycle with birds as amplifying hosts and ornithophilic mosquitoes as vectors. The virus generally only causes a mild arboviral disease in humans. However, some of the less common symptoms are meningoencephalitis in immunocompromised patients or neurologic disorders (e.g. diopathic facial paralysis).

After the first detection in 2010, two citizen science based USUV surveillance systems were established: a dead bird screening program and a reporting system for conspicuous birds. In addition, data from an existing monitoring program of the bird abundance in gardens was analysed to understand USUV epidemiology in Germany.

A high forecasting accuracy for the spatial-temporal risk of USUV circulation was observed for the data from the dead bird screening program and the reporting system for conspicuous birds. However, as expected, the accuracy significantly decreased for larger time periods. Nevertheless, the forecasting horizon is probably long enough to inform critical decision making in space and time, e.g. blood donation testing. In contrast, annual bird abundance data gave less accurate results.

This study highlights that near real-time forecasting using citizen science-based data has the potential to become an important tool for public health authorities during infectious disease outbreaks.

## Cluster Analysis with German Antimicrobial Resistance (AMR) Data for Humans and Food-producing Animals

Beneditta SUWONO[1,2], Tim ECKMANNS[2], Heike KASPAR[3] and Bernd-Alois TENHAGEN[1]

[1]German Federal Institute for Risk Assessment, Berlin, Germany
[2]Robert Koch Institute, Berlin, Germany
[3]Federal Office of Consumer Protection and Food Safety, Berlin, Germany

*Keywords: Antimicrobial Resistance, One Health, Escherichia coli*

German One Health Initiatives (GOHI) has been initiated within German National Action Plan on AMR (DART 2020). This study aims to cluster AMR data from German national surveillance and monitoring systems using data on Escherichia coli from 2014 to 2017. We used data from the Antibiotic Resistance Surveillance (ARS) for humans, Zoonosis-Monitoring for non-clinical food-producing animal isolates and GERM-Vet for clinical animal isolates. Human data originated from outpatients, general wards and intensive care units. Food-producing animals data were stratified by animal type and clinical vs. non-clinical origin. We included 3 human origins and 38 animal origins (e.g. pigs-clinical, etc.) and four antibiotics that were frequently tested in all systems: ampicillin, cefotaxime, ciprofloxacin and gentamicin which resulted in 16 resistance combinations. Using hierarchical clustering in R (Euclidian, Average), we detected three different clusters based on the resistance combinations in isolates from each origin. Clinical human isolates clustered together. The cluster was closely related with isolates from pigs (e.g. weaners, clinical piglets). All isolates that having low resistance rates (<20%) against these four antibiotics clustered together (mostly non-clinical food-producing animals). All poultry isolates except broilers from organic farms grouped together with clinical isolates from pigs and bovines <1 year. Further analyses to better understand the clusters are necessary.

## Session 7: Novel Methods

**October 17, 2019**
**11:00 – 12:30**

**Room: Steglitz**
**Chairs: Imke Steffen and Sascha Al-Dahouk**

## An antique origin of measles

Ariane Düx[1], Sebastian Lequime[2], Livia Patrono[1], Baptiste Prepoint[1], Markus Ulrich[1], Jasmin Schlotterbeck[1], Jan Gogarten[1], Kevin Merkel[1], Annette Mankertz[3], Navena Widulin[4], Fabian Leendertz[1], Thomas Schanlke[5], Philippe Lemey[2], Sébastien Calvignac-Spencer[1]

[1] Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institute, Berlin, Germany
[2] KU Leuven Department of Microbiology and Immunology, Rega Institute, Laboratory of Clinical and Evolutionary Virology, Leuven, Belgium
[3] National Reference Center Measles, Mumps, Rubella, Robert Koch Institute, Berlin, Germany.
[4] Berlin Museum of Medical History at the Charité, Berlin, Germany
[5] Berlin Museum of Medical History at the Charité, Berlin, Germany

*Keywords: measles, time dependent rates, ancient DNA*

Measles morbillivirus (MeV) is a major human pathogen. Despite its global impact, its origin remains controversial. MeV likely originated in cattle; its closest known relative is Rinderpest morbillivirus (RPV). Historical considerations suggest two preconditions for this host switch: 1. Cattle domestication (>10,000 years ago) which considerably increased exposure to the reservoir, and 2. Sufficiently large human populations to support endemism despite lifelong immunity post infection (since ~5,000 years ago). These considerations favor an ancient origin, but are contradicted by attempts at molecular dating which provide much more recent estimates, as late as the 12th century. Such discrepancies are notorious for evolutionary timescales of RNA viruses. The molecular clock of RNA viruses appears to tick faster when rates are determined by tip calibration rather than by deeper node calibration. Dates of deep divergence events determined from tip calibration often appear unrealistically recent. Addressing this problem, we extended the observation time frame by using the oldest human RNA virus genome sequenced to date, a 1912 archival specimen, and applied a novel time dependent-rate epoch model to reassess the date of MeV emergence. Our results suggest an antique origin of MeV with a MeV/RPV divergence date around 340 years B.C. (95% HPD -1050 to +285). This date is much more ancient than all previous molecular clock based estimates and consistent with historical considerations.

## Rabies Virus Cell Tropism in 3D – comparison of attenuated, lab and field strains

Madlin Potratz, Luca Zaeck, Stefan Finke

Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institute (FLI), Greifswald-Insel Riems, Germany

Rabies virus (RABV) is a highly neurotropic virus that spreads through the peripheral and central nervous system via trans-synaptic spread by budding and entry at synaptic membranes. In the brain, infection of not synaptically connected cells is limited, making RABV a powerful tool for trans-synaptic mapping of connected neurons.
Restriction of RABV cell tropism to brain neurons may be determined through highly regulated release of RABV virions at synaptic membranes and strong innate immune responses by infected astrocytes may lead to abortive infection. Indeed, the latter has been demonstrated for lab adapted RABV strains. However, the potential of highly virulent field RABVs to infect non-neuronal cells in vivo and its contribution to virulence is unclear.
Here, using 3D immunofluorescence imaging of solvent-cleared brain tissue slices, we compared the cell tropism of attenuated SAD L16, lab-adapted CVS-11 and two field RABV derived from dog and fox. Independent of the degree of attenuation or lab adaptation, all viruses mainly infected neurons. However, field RABV infections also led to strong virus protein expression in non-neuronal astrocytes. In contrast, astrocyte infection was not detectable for lab-adapted RABVs. This supports a model in which efficient replication of field RABV in astrocytes represents a backup immune evasion mechanism that may prevent local antiviral responses in a situation where virus release in not entirely restricted to synaptic membranes.

## Three-dimensional co-cultivation of cells from different species and tissue origin in a novel bioprinted array as a diagnostic tool for virus detection

Robert Koban[1], Markus Neumann[1], Franziska Schwarz[1], Tobias Lam[2], Lutz Kloke[2], Heinz Ellerbrok[1]

[1]Robert Koch-Institute
[2]Cellbricks GmbH Berlin

*Keywords: 3D cell culture, multicellular array, bioprinting, virus diagnostic tool*

Monolayer cell cultures represent a functional approach for virus propagation and characterization. However, the biological characteristics of virus-host interaction may be altered compared to an in vivo infection and not every virus can be grown on a cellular monolayer. 3D cell cultures can overcome these problems, as cells show a more in vivo-like behavior and therefore are a promising option to propagate and characterize viruses difficult to cultivate using conventional methods. For this reason, we aimed at establishing a cell culture platform with a bioprinted 3D matrix for virus diagnostics and characterization. To establish a 3D cell culture array, cells of different tissues and host origin were cultured on 3D bioprinted matrices and were characterized for their growth behavior. The cells were cultivated as 3D co-cultures for at least one week and then were infected with different zoonotic viruses including Cowpox virus and Puumala virus which differ in their host range and tissue tropism. The transparent matrix enabled an easy monitoring of infection even by live-cell microscopy.
In conclusion, we established a standardized and easy-to-handle 3D bioprinted cell culture array suitable for easy and sensitive monitoring and subsequent characterization of tissue tropism and host range of different DNA and RNA viruses. The diagnostic tool will be further characterized for sensitivity and preliminary in vivo-related characterization of diagnostic samples with unknown viruses.

## Differential efficacy of novel antiviral substances against Cowpox virus (CPXV) in 3D and monolayer cell culture

Markus Neumann, Robert Koban, Philipp Nelson, Heinz Ellerbrok

Robert Koch-Institute, Centre for Biological Threats and Special Pathogens, ZBS1: Highly Pathogenic Viruses, Berlin, Germany

*Keywords: 3D cell culture, host-directed therapy, cowpox virus, EGFR*

At present only a few antivirals against zoonotic CPXV are available. Repurposing of approved drugs can overcome this shortage. Most aspects of virus biology including screening of antivirals are studied in vitro in 2D cell cultures. However, the biology of these cultures differs from the in vivo situation in infected tissues. 3D models may reflect more realistically the in vivo infection.
Therefore, we established a 3D infection model for CPXV with primary human keratinocytes for screening of different host-directed molecules as potential novel antiviral substances.
Small molecule EGFR-inhibitors with different modes of action, as well as an EGFR-inhibiting antibody were tested in 3D culture for their antiviral activity and efficacy was compared with the corresponding 2D approaches. In contrast to the virus-directed antiviral tecovirimat, the host-directed inhibitors afatinib and cetuximab were approx. 100-fold more efficient against CPXV infection in 3D culture, similar to results previously observed with gefitinib. In summary, inhibition of EGFR-signaling downregulates virus replication comparable to established virus-directed antiviral therapeutics. However, in vitro efficacy of host-directed antivirals is seriously affected by 3D cultivation of the cells whereas virus-directed substances inhibit virus replication independent of the cultivation system. The results suggest that screening of such drugs in standard 2D culture might underestimate the potential of antivirals.

## A specialized thin agar method (STAM) to study electric signaling in bacterial biofilms

J. Assmann, C. Schaudinn, S. Bürge, M. Laue, B. Walther

ZBS 4 Spezielle Licht- und Elektronenmikroskopie, Robert Koch-Institute, Berlin, Germany

*Keywords: bacterial biofilm, microcolony, electric signaling, membrane potential*

Prevention or even eradication of bacterial biofilms in medical settings is a persistent issue worldwide. Besides quorum sensing mediated by small signal molecules, bacteria in biofilms also communicate via electric signaling in form of potassium ions. A gate protein (TrkA) directing potassium ion channels was recently identified as modulator of electric signal waves, supporting nutrient supplies to reach inner biofilm layers. The successful survival of a biofilm, including those of zoonotic relevance, may be associated with these long-ranging electric signals.
To visualize electric oscillation in biofilms, which is probably induced by membrane depolarization, a specialized thin agar method (STAM) has been developed: µ-Slides (ibidi GmbH) are spotted with 10 µl minimal medium and covered with a thin layer of agar (1.5 mm thickness). Afterwards, the tip of an Ø 0.25mm insect needle was used to inoculate bacterial strains underneath the agar layer. After 5.5 h of incubation at 37 °C, a fluorescent dye indicating membrane depolarization (Thioflavin T) was carefully added. Subsequently, live cell imaging (12 h at 30 °C) was performed using confocal laser scanning microscopy (CLSM).
Here we present preliminary results of a reliable, inexpensive and easy to use STAM assay. Vertical colony expansion limited eased long-term visualizing of electric communication via CLSM in biofilms consisting of Bacillus subtilis and Staphylococcus epidermidis, including the nosocomial strain RP62A.

## An in vitro system to study heteroresistance and metabolic host interaction on mature tissue cysts of *Toxoplasma gondii*

C. Christiansen[1], J. Scholz[1], F. Seeber[2], M. Laue[3], M. Blume[1]

[1] Junior Group 2: Metabolism of Microbial Pathogens, Robert Koch-Institute, Berlin, Germany
[2] FG 16: Mycotic and Parasitic Agents and Mycobacteria, Robert Koch-Institute, Berlin, Germany
[3] ZBS 4: Advanced Light and Electron Microscopy, Robert Koch-Institute, Berlin, Germany

*Keywords: bradyzoites, phenotypic heterogeneity, metabolism, host-parasite interaction*

Persisting stages of T. gondii are a key source of transmission and acute toxoplasmosis but cannot be targeted with currently available treatments.
Because access to mature cysts is limited to in vivo models the mechanistic basis of this resistance is hard to study and identification of compounds that target tissue cysts efficiently remains challenging. To address both problems we optimized the generation of mature T. gondii cysts in vitro. To this end we infected terminally differentiated human myotubes as natural host cells with a wide range of parasite strains. Indeed, these cells support long-term culture of cysts, including type I RH parasites, without interfering growth of tachyzoites.
Confirming their phenotypic similarity to in vivo cysts, our cysts lack expression of the tachyzoite antigen SAG1, develop pepsin resistance after three weeks and survive one week-long treatments with high doses of Pyrimethamine, Sulfadiazine, the quinolone HDQ and bumped-kinase inhibitors against CDPK1. Our EM-based ultrastructural analyses indicate broad distribution of polysaccharide stores, parasite packaging densities and proliferation within the same cyst, suggesting a heteroresistance mechanism against these compounds. Interestingly, host cell mitochondria associate with the vacuoles of type 2 parasites in these cells. This contrasts observations in fibroblasts and we are currently investigating implications on lipid uptake using mass spectrometry-based metabolomics.
Summarized, we identified a human host cell line that can be used to raise T. gondii tissue cysts that are functionally similar to in vivo cysts. Our method will facilitate future studies on bradyzoite biology and enable the identification of bradyzoidal compounds.

# Plenary Session II

**October 17, 2019**
**14:30 – 16:00**

**Room: Ballsaal**
**Chair: Martin H. Groschup**

# Animal tuberculosis: the challenge of controlling a global multi-host infection

Christian Gortázar Schmidt[1]

[1]Institute for Game and Wildlife Research, Universidad de Castilla, Spain

*Keywords: Epidemiology and disease control; Mycobacterium bovis; Wildlife; Zoonotic tuberculosis*

This presentation will mainly address animal tuberculosis (zoonotic tuberculosis, TB), an often neglected zoonosis caused by infection with *Mycobacterium bovis* and other closely related members of the *M. tuberculosis* complex (MTC). This is a global problem affecting human health and livelihood, animal health and welfare, and conservation. Despite MTC being a typical multi-host pathogen, in most industrialized countries, existing TB control schemes are restricted to the target domestic host, cattle. In developing countries, where the zoonotic risk is highest, TB control schemes in livestock are generally incipient or even absent. In both situations, examples of holistic TB control approaches, i.e. those addressing all suitable domestic and wild maintenance hosts and exploring the benefits of all suitable TB-control tools, remain exceptional. If nothing changes, animal TB will remain one of the biggest health challenges of the 21st century. Science contributes innovative approaches for TB control, with new insights coming from several fields including the global fight to control human TB and new vaccine candidates; novel molecular epidemiology results at the cell, host and population scale; and information on animal ecology, epidemiology and modeling in multi-host systems. Insights and tools derived from research on *M. bovis* can eventually benefit the control of other zoonotic diseases with a strong wildlife component.

## Situation of leishmaniosis in Europe from humans to wildlife

Laia Solano-Gallego[1]

[1]Department of Animal Medicine and Surgery, Autonomous University of Barcelona, Spain

*Keywords: Leishmaniosis, Leishmania infantum, human, pets and wildlife*

Leishmaniosis (or 'leishmaniasis') is a complex of mammalian sandfly-borne diseases caused by diphasic protozoans of the genus *Leishmania*. In Europe, the most common clinical entities reported are cutaneous leishmaniosis (CL) and visceral leishmaniosis (VL) due to *Leishmania infantum* infection. Although human leishmaniosis is considered a disease of the developing world, *L. infantum* infection is currently spreading northwards, outbreaks are occurring in endemic areas, and foci of the disease are appearing in previously non-endemic countries, not necessarily associated to HIV as it occurred in the past. In Europe, clinical disease in humans is much lower than asymptomatic infection that is by far more frequent. As in humans, *Leishmania* infection in dogs is manifested as a subclinical infection, self-limiting disease or a severe and fatal disease and immune responses are extremely important in disease development. Non-sand fly modes of transmission have also been described but their role in the natural history and epidemiology of *L. infantum* infection remains unclear. Proven modes of non-sand fly transmission in dogs include infection through transfused blood products from blood donors, which are carriers of infection, vertical and venereal transmission. The role of other domestic and wild life animals in the epidemiology of leishmaniosis remains controversial. High rates of subclinical infection have been reported when testing for leishmaniosis using molecular techniques in cats, wild canids and hares. Transmision of *L. infantum* to *Phlebotomus* sandflies has been reported in cats and hares.

## Session 8: Pathogen-Cell Interaction

**October 18, 2019**
**09:30 – 11:00**

**Room: Zehlendorf**
**Chairs: Anja Lührmann and Stephan Ludwig**

## Immunogenicity and fetoprotective capacity of recombinant MVA Virus expressing Zika Virus E and prM/M proteins in a pregnant mouse model

Dominik Forster, Jan Hendrik Schwarz, Gerd Sutter, Asisa Volz

Institute for Infectious diseases and zoonoses, Chair for Virology, LMU Munich, Germany

*Keywords: Zika virus, vaccination, pregnancy, ultrasound, MVA*

Zika Virus (ZikV) poses a major challenge as zoonotic pathogen. It has been recently confirmed to cause Guillain-Barre syndrome, meningoencephalitis and in addition, the infection of pregnant women could result in fetal abnormalities including microcephaly and spontaneous abortion. We still know little about the mechanisms by which ZikV infects the fetus during pregnancy. Preliminary data suggest that ZikV may bypass the placenta to reach the fetus. Thus, preventive options appropriate for pregnant women make special demands in activating a balanced immunity comprising the maternal, placental and fetal response in an equalized manner to avoid overly strong reactions.
Modified Vaccinia virus Ankara (MVA) serves as a poxvirus vector in the development of new vaccines. Here, we tested a recombinant MVA expressing ZikV E and prM/M proteins (MVA-prME) and analyzed the protective capacity in a mouse pregnancy model.
In mock vaccinated control mice, challenge resulted in maternal ZikV specific illness and infection of the placenta and the developing fetuses. Here, ultrasound scanning revealed modified blood flow in umbilical circulation or death. In contrast, MVA-prME immunized mice did not show any effect of ZikV challenge infection for fetal or maternal health.

## Impact of polymorphisms in the MERS-coronavirus spike protein and its receptor DPP4 on viral entry into host cells

Hannah Kleine-Weber[1,2], Simon Schröder[3,4], Nadine Krüger[5,6], Khaled R. Alkharsah[7], Marcel A. Müller[3,4], Christian Drosten[3,4], Stefan Pöhlmann[1,2] and Markus Hoffmann[1]

[1]Infection Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany
[2]Faculty of Biology and Psychology, University Göttingen, Wilhelm-Weber-Str. 2, 37073 Göttingen, Germany
[3]Institute of Virology, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany
[4]German Centre for Infection Research, associated partner Charité, Germany
[5]Institute of Virology, University of Veterinary Medicine Hannover, 30559 Hannover, Germany
[6]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, 30559 Hannover, Germany
[7]Institute for Research and Medical Consultations (IRMC), University of Dammam, Dammam 31441, Kingdom of Saudi Arabia

*Keywords: MERS-coronavirus, Spike glycoprotein, TMPRSS2, DPP4, Polymorphisms*

Middle East respiratory syndrome coronavirus (MERS-CoV) causes a severe respiratory disease first recognized in 2012 in Saudi Arabia. MERS-CoV host cell entry is driven by the viral spike protein (S) and relies on S protein activation by the host cell protease TMPRSS2 and on binding of MERS-S to its cellular receptor, dipeptidyl-peptidase 4 (DPP4). At present, it is unclear how polymorphisms in MERS-S and DPP4 impact MERS-S activation and receptor binding.
We analyzed public databases for MERS-S polymorphisms located within or adjacent to the S1/S2 and S2' cleavage sites. Moreover, we searched for polymorphisms in DPP4, which alter residues that interact with amino acids in the MERS-S receptor binding site. We found that polymorphism R748C, located in the S1/S2 site of MERS-S, reduces S protein processing by furin in MERS-S expressing cells and subsequent MERS-S-driven, TMPRSS2-dependent entry into target cells. In addition, we showed that polymorphism T746K increased S-protein processing by TMPRSS2. Finally, we found that polymorphisms K267E, K267N and A291P in DPP4 reduce MERS-S binding to DPP4 and MERS-S-driven entry. Collectively, our results demonstrate that certain polymorphisms within MERS-S and DPP4 are not compatible with robust viral entry into host cells and might thus alter viral spread and disease development.

## A single amino acid substitution in the Ebola virus glycoprotein reduces the dependence of host cell entry on cellular cysteine proteases

Markus Hoffmann, Svenja Kaufmann, Carina Fischer, Wiebke Maurer, Stefan Pöhlmann

Infection Biology Unit, German Primate Center, Göttingen, Germany

*Keywords: Ebola virus, glycoprotein, cathepsin, in-vitro evolution*

Background and objectives
Ebola virus (EBOV) infection can cause a life-threatening disease with high case-fatality rates. After virus attachment, EBOV is taken up into endosomal vesicles where the viral glycoprotein (GP) is proteolytically primed by cellular cysteine proteases (cathepsin (Cat) B and L), a step that is required for subsequent receptor engagement and membrane fusion. Because of their important role in the EBOV lifecycle, host factors like CatB/L are targets for antiviral therapy. We sought to analyze whether EBOV-GP has the potential to adapt to low levels of CatB/L activity.

Materials and methods
We passaged a replication-competent vesicular stomatitis virus expressing EBOV-GP (VSV-EBOV) on cells pre-treated with CatB/L inhibitor. The EBOV-GP gene was further sequenced and mutations were analyzed for their impact on CatB/L-dependence of EBOV-GP-driven host cell entry.

Results
Passaging on inhibitor-treated cells awarded VSV-EBOV with a replicative benefit over the unpassaged virus under conditions of low CatB/L activity. Further, a single amino acid exchange in EBOV-GP was identified that reduces the dependence of EBOV-GP-driven cellular entry on CatB/L activity in single-round transduction experiments.

Conclusion
We identified a point mutation that reduces CatB/L-dependence of EBOV-GP-driven host cell entry and further show the ability of EBOV-GP to adapt to conditions of limiting CatB/L activity.

## Enhanced replicative capacity and anti-immune activity of epidemic recombinant MERS-Coronavirus

Simon Schroeder[1], Christin Mache[2], Doreen Muth[1], Victor M. Corman[1], Ronald Dijkman[3], Ziad Memish[4], Abdulla M. Assiri[5], Thorsten Wolff[2], Marcel A. Müller[1], Christian Drosten[1]

[1]Institute of Virology, Charité-Universitätsmedizin Berlin, Berlin, Germany
[2]Unit 17, Influenza and other Respiratory Viruses, Robert Koch Institut, Berlin, Germany
[3]Institute of Virology and Immunology, Bern & Mittelhäusern, Department of Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland
[4]College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia, Ministry of Health, Riyadh, Kingdom of Saudi Arabia
[5]King Abdulaziz University, Faculty of Science, Chemistry Department Jeddah, Kingdom of Saudi Arabia

Abstract:
Middle East respiratory syndrome coronavirus (MERS-CoV) is a widespread virus in dromedary camels that can cause life-threatening pneumonia in humans. In or before 2014, a recombination event between two distinct phylogenetic clades (groups 3 and 5) formed in a new recombinant virus clade (MERS-$CoV_{NRC}$). While the clade became widely endemic and caused large nosocomial outbreaks in Saudi Arabia and South Korea, it remains unclear whether the recombinant virus shows signs of increased virulence or transmissibility. Here we performed a comprehensive in-vitro comparison of parental and recombinant strains based on rare virus isolates from Saudi Arabia.
We generated 23 MERS-CoV isolates from human respiratory samples obtained between 2014 and 2015. The isolates belong to clade 3 (N=7), clade 5 (N=8), as well as MERS-$CoV_{NRC}$ (N=8). Using infection assays with at least two representative virus isolates per group, we found replication of MERS-$CoV_{NRC}$ to be significantly increased over the parental strains in in-vitro and ex-vivo lung models. On primary human lung epithelial cells as well as permanent cell lines Calu3 (lung) and Caco2 (colon) cells, MERS-$CoV_{NRC}$ isolates replicated faster and reached final viral titers up to 10-fold higher than parental clade 3 and 5 isolates. Transcriptional profiling by real-time RT-PCR showed that several key immune genes (IFNb1, CCL5, IFNL1) were significantly less induced in lung cells infected with MERS-$CoV_{NRC}$ than parental groups. MERS-$CoV_{NRC}$ further showed increased resilience against interferon (IFN) pre-treatment of Calu-3 cells and maintained 10-fold higher replication under low and high concentrations of IFN. Reduced immune activation combined with enhanced virus replication and IFN resilience may have facilitated the epidemiological dominance of MERS-$CoV_{NRC}$ on the Arabian Peninsula. Future work will explore if the recombination event itself or if other (adaptive) mutations after the recombination event are linked to the observed phenotype.

## Structure of a functional cap-binding domain in Rift Valley fever virus L protein

Nadja Gogrefe, Sophia Reindl, Stephan Günther, Silke Olschewski, Maria Rosenthal

Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

*Keywords: Bunyavirales, viral transcription, cap-snatching mechanism*

Rift Valley fever virus (RVFV) belongs to the family of Phenuiviridae within the order of Bunyavirales. The virus may cause fatal disease both in livestock and humans, and therefore, is of great economical and public health relevance. In analogy to the influenza virus polymerase complex, the bunyavirus L protein is assumed to bind to and cleave off cap structures of cellular mRNAs to prime viral transcription. However, even though the presence of an endonuclease in the N-terminal domain of the L protein has been demonstrated for several bunyaviruses, there is no evidence for a cap-binding site within the L protein. We solved the structure of a C-terminal 117 amino acid-long domain of the RVFV L protein by X-ray crystallography. The overall fold of the domain shows high similarity to influenza virus PB2 cap-binding domain and the putative non-functional cap-binding domain of reptarenaviruses. Upon co-crystallization with $m^7$GTP, we detected the cap-analogue bound between two aromatic side chains as it has been described for other cap-binding proteins. We observed weak but specific interaction with $m^7$GTP rather than GTP in vitro using isothermal titration calorimetry. The importance of $m^7$GTP-binding residues for viral transcription was validated using a RVFV minigenome system. In summary, we provide structural and functional evidence for a cap-binding site located within the L protein of a virus from the Bunyavirales order.

## Single cell assay for DNA uptake in *Campylobacter jejuni* - a tool for monitoring the adaptive potential of the food-borne pathogen

Julia Golz, Kerstin Stingl

Bundesinstitut für Risikobewertung

*Keywords: natural transformation, genetic diversity, fitness, reduction strategies, food safety*

**Background**
Natural transformation can increase genetic diversity and, therefore, the adaptive potential of Campylobacter jejuni. In order to develop reduction strategies, it is important to understand the mechanisms of competence development and DNA uptake in C. jejuni.

**Approach**
To visualize DNA uptake C. jejuni was incubated with fluorescently labelled DNA and analyzed on a single-cell level. The ratio of competent cells was calculated. We genetically constructed mutants lacking homologues of either pilQ, comE or comEC, which were shown to be essential for DNA uptake in other bacteria.

**Results**
The established single-cell assay provides the opportunity to analyze Campylobacter competence development. We visualized DNA uptake in C. jejuni using the developed single cell assay. Approximately 20 % to 30 % of C. jejuni cells took up fluorescently labelled C. jejuni DNA under standard conditions and showed DNase resistant fluorescent foci. The ΔpilQ mutant was deficient for DNA uptake, as expected from the predicted role as outer membrane DNA channel in Neisseria. The ΔcomE and ΔcomEC mutants showed wild-type levels of DNA uptake (into the periplasm). Although ComE is known as a DNA-binding protein facilitating DNA uptake, our results indicate no essential role in DNA uptake in C. jejuni. Parallel transformation experiments with the ΔcomEC mutant showed no transformation activity. This is in accordance with ComEC being the inner membrane channel.

**Conclusions**
The developed single cell DNA uptake assay provides visualization of DNA uptake. Two mutants showed defects in natural transformation. These mutants will be further analyzed, in cooperation with members of the PAC-CAMPY consortium, for their adaptive potential in vivo and their ability to form biofilms.

## Session 9: New and Re-Emerging Zoonotic Diseases II

**October 18, 2019**
**09:30 – 11:00**

**Room Steglitz**
**Chairs: Stephanie Becker and Martin H. Groschup**

## The cowpox virus reservoir – are host-species specific strains the key?

S. Weber[1], K. Jeske[2], F. Pfaff[1], R.G. Ulrich[2], M. Beer[1], D. Hoffmann[1]

[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[2] Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany

*Keywords: CPXV, Reservoir host, bank vole, common vole, CAM*

Cowpox virus (CPXV) strains are endemic in Western Eurasia– and the incidence of human CPXV infection is increasing. Especially voles are suspected to be the major reservoir host species. Seroprevalence studies from continental Europe and UK in different voles have led to this assumption. While experimental studies verified seroconversion upon exposure in bank voles (Myodes glareolus) and common voles (Microtus arvalis), viral excretion was detected only in common voles by now.
Here we characterized a novel CPXV isolate derived from a bank vole sample in comparison to a genetically related isolate originating from a Cotton-top tamarin (Saguinus oedipus). Both isolates build a novel clade within the CPXV-species.
In vitro characterization on Vero cells and the chorioallantoic membrane yielded no differences compared to the reference strain Brighton Red. For in vivo analyses, both bank and common voles were inoculated intranasally and monitored for disease, viral DNA shedding in nasal/buccal swabs and seroconversion. In contrast to previously reported studies, robust viral shedding for both isolates was observed in bank voles, but not in common voles. In addition, bank voles infected with the cotton-top tamarin isolate experienced a marked weight loss.
Our findings suggest that CPXV isolates are adapted to distinct reservoir hosts. Interestingly, the phylogenetic analyses reflected the phenotypical difference by a diverse clade assignment of the new isolates.

## Phenotypes of MERS-CoV infections in human lung explant cultures

C. Mache[1], S. Schroeder[2], D. Fatykhova[3], A. Hocke[3], S. Hippenstiel[3], M. Müller[2], C. Drosten[2], T. Wolff[1]

[1]Robert Koch Institut, Unit 17, Influenza and Other Respiratory Viruses, Berlin, Germany
[2]Charité - Universitätsmedizin Berlin, Department of Infectious Diseases and Respiratory Diseases
[3]Charité - Universitätsmedizin Berlin, Institute of Virology

*Keywords: MERS-CoV, lung explant cultures, human lung*

Introduction
MERS-CoV can be acquired as a zoonotic infection upon contact with infected dromedary camels. Limited human-to-human transmission is observed predominantly in the context of nosocomial outbreaks that are accompanied by a high case-fatality ratio. MERS-CoV is divided into 3 clades (A-C) with viruses from clade B dominating in the Arabian Peninsula. Clade B viruses are further divided into 5 lineages. In 2015 a novel recombinant subclade (NRC-2015) originating from parental strains from lineages 3 and 5 was identified, which became predominant among MERS-CoV circulating in Saudi Arabia. So far it is unclear to what extent the virus lineages compare regarding their zoonotic potential as well as to their potential to infect the lower respiratory tract of humans.
Materials & Methods
Tumor-free human lung tissue and polarized Calu-3 cell cultures were infected with virus isolates belonging to MERS-CoV subclade NRC-2015 and viruses belonging to the parental lineages 3 and 5 as well as the prototypic EMC strain.
Results
In lung explant cultures the recombinant subclade virus propagated up to one log-level higher compared to the parental lineage 3 and 5 viruses, as well as the prototypic EMC. Comparable results were observed on polarized Calu-3 cells.
Conclusion
The results indicate that a recombination event led to the appearance of the NRC-2015 lineage that has apparently increased adaptation to the human respiratory tract.

## Establishment of a reverse genetics system for the cell-culture-adapted hepatitis E virus genotype 3c-strain 47832c

J. Scholz[1], A. Falkenhagen[1], C. Bächlein[2], R. Johne[1]

[1] German Federal Institute for Risk Assessment, Berlin, Germany
[2] University of Veterinary Medicine Hannover, Institute of Virology, Bünteweg 17, 30559 Hannover, Germany

*Keywords: Hepatitis E virus, zoonosis, reverse genetic system, cell culture, A549 cells, site-directed mutagenesis*

Hepatitis E virus (HEV) is a zoonotic pathogen, which can cause hepatitis in humans. The study of HEV is hampered by the lack of efficient cell culture systems. However, a few HEV strains replicate stably in cell culture, which are therefore often used for basic and applied studies. One of those strains, HEV47832chas been originally isolated from a chronically infected transplant patient in Germany. It represents a typical genotype 3c strain, but contains a unique insertion in its ORF1, which might be responsible for efficient in-vitro growth.
We describe the development of a reverse genetics system for this virus. After cloning the complete virus genome and subsequent transfection of in-vitro transcribed viral RNA, no infectious virus could be generated. Comparison of the plasmid sequence with two independently generated virus genome sequences identified point mutations. Through exchange of those mutations, infectious virus could be generated. The final protocol included co-transfection of BSR/T7 cells with the circular full-length plasmid and two helper plasmids expressing capping enzymes followed by passages on A549/D3 cells. Virus replication was demonstrated by immunofluorescence and the correct 3'-end of the virus genome was verified by RT-PCR.
Currently, the generated virus is compared with the original strain regarding growth kinetics and site-directed mutations are introduced. The system will enable targeted mutagenesis of this widely used HEV strain for basic and applied applications.

## Identification of animal reservoirs of Fort Sherman virus, a New World zoonotic orthobunyavirus

Edmilson F. de Oliveira Filho[1], Roberto Franke[2], Ianei Carneiro[2], Jan Felix Drexler[1], Carlo Fischer[1]

1 Charité – Universitätsmedizin Berlin
2 Universidade Federal da Bahia

*Keywords: Orthobunyaviruses, Arbovirus, horses, zoonoses*

Orthobunyaviruses are arthropod-borne viruses associated with zoonotic transmission and animal disease worldwide. Fort Sherman virus (FSV) was isolated in 1985 from a US soldier based in Panama with acute febrile disease. The virus was antigenically related to Maguari and Cachey Valley viruses and assigned to the Bunyamwera serogroup, however, back then, no other vertebrate host could be associated with the virus. Similarly, potential FSV vectors remained unknown. Here, we used a broadly reactive and highly sensitive nested RT-PCR targeting the L gene to investigate a total of 2,875 domestic animals from Brazil sampled during 2013-2016 for orthobunyavirus RNA, including cattle, sheep, goat, horses, dogs and cats. One horse from the state of Bahia was viremic and the virus classified as FSV upon typing of the screening PCR amplicon, at 97.3% translated amino acid identity to the FSV prototype sequence obtained from the soldier. The virus was successfully isolated from horse serum in Vero E6/7 cells. Replication studies in different invertebrate cell lines, full genome characterization and serological studies are ongoing and will be presented. Our findings highlight the occurrence of FSV across a geographic range exceeding 3000 kilometers and surprising genomic conservation across a timespan exceeding 30 years, contributing to our understanding of the epidemiology of Latin American orthobunyaviruses, including possible animal reservoirs of FSV.

## Mammalian hepatitis delta virus without hepadnavirus coinfection in the neotropical rodent Proechimys semispinosus

Sofia Paraskevopoulou[1], Fabian Pirzer[1], Julian Schmid[2], Victor Corman[1], Nora Goldmann[3], Simon Schroeder[1], Lina Theresa Gottula[1], Andrea Rasche[1], Doreen Muth[4], Jan Felix Drexler[1], Alexander Christoph Heni[5], Georg Joachim Eibner[1], Rachel A. Page[6], Terry C. Jones[1], Marcel Alexander Müller[1], Dieter Glebe[7], Simone Sommer[3], Christian Drosten[1]

[1] Institute of Virology, Charité Universitätsmedizin Berlin
[2] Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm
[3] Institute of Medical Virology, Justus Liebig University of Giessen
[4] State Office for Health and Social Affairs (LAGeSo)
[5] Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm
[6] Smithsonian Tropical Research Institute
[7] Institute of Medical Virology, Justus Liebig University of Giessen

*Keywords: Hepatitis delta virus, Proechimys semispinosus, Hepatitis B virus, coinfection, RNA virus evolution, neotropical rodent*

Hepatitis delta virus is a satellite virus of Hepatitis B virus, unrelated to any other taxonomic group of RNA viruses. It is a singular case in animal virology for which no consensus evolutionary explanation exists. We present the first mammalian deltavirus that does not occur in humans, found in the neotropical rodent Proechimys semispinosus. The rodent deltavirus is highly distinct, showing a common ancestor with deltavirus in snakes. Reverse genetics on a tandem minus strand cDNA genome copy under the control of a CMV promoter confirm autonomous genome replication in transfected cells, with initiation of replication from the upstream genome copy and dependence of replication on expressing the small delta antigen. Large delta antigen is not expressed and the farnesylation motif critical for HBV interaction in HDV is absent from the equivalent large antigen region in the rodent deltavirus genome. There is no evidence for coinfection with an HBV-related hepadnavirus based on virus detection and serology in any deltavirus positive animal. No other coinfecting agents were detected by RNAseq studies of 120 wild-caught animals. Presence of virus in blood and pronounced detection in reproductively active males suggest horizontal transmission linked to competitive behavior. Our study establishes a non-human mammalian deltavirus that occurs as a vertically-transmitted infection, is likely cleared by immune response, is not focused in the liver, and does not require HBV coinfection.

## Arenavirus persistence in *Mastomys natalensis*, the natural rodent reservoir of Lassa virus

C. Hoffmann[1,2], E. Pallasch[1,2], S. Bockholt[1,2], D. M. Wozniak[1,2], J. Müller[1,2], L. Oestereich[1,2], S. Günther[1,2]

[1]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany
[2]German Center for Infection Research (DZIF), Partner site Hamburg, Germany

*Keywords: Lassa virus, LASV, Mastomys natalensis, arenaviruses*

Lassa virus (LASV) is a single-stranded RNA virus belonging to the Arenavirus family and is endemic in several regions in Western Africa. This rodent-borne pathogen can cause a severe haemorrhagic fever in humans leading up to 5000 deaths per year. Its natural host is the natal multimammate rat *Mastomys natalensis*, a commensal rodent that inhabits large parts of Sub-Saharan Africa.
So far little is known about the pathogenesis in humans or the viral dynamics in the rodent host. Our aim is to characterize the course of infection and viral dynamics of LASV and related arenaviruses in Mastomys natalensis. In this study we conducted a series of transmission and infection experiments using the closely related Morogoro virus (MORV) as a surrogate for LASV infection in the rodent host.
Mastomys natalensis were infected with MORV at different timepoints using different infection routes. Blood, organs and urine were sampled in frequent intervals up to four months post infection and viral RNA levels and antibody-presence were analysed.
We could show that the age of the host plays a crucial role for the development of viral persistence in Mastomys natalensis. Contact with MORV within the first two weeks after birth leads to a chronic infection whereas older animals only show transient viremia followed by seroconversion. Furthermore, chronically infected individuals remain viremic, despite the presence of antibodies, and shed infectious virus throughout their lifetime.

## Session 10: Antimicrobial use and resistance

**October 18, 2019**
**09:30 – 11:00**

**Room Ballsaal**
**Chairs: Birgit Walther and Robin Köck**

## Vitamin C alleviates *Campylobacter jejuni* induced acute enterocolitis in a clinical model for acute campylobacteriosis

Stefan Bereswill, Soraya Mousavi, Ulrike Escher, Anna-Maria Schmidt, Markus M. Heimesaat

Institute of Microbiology & Infection Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany; PAC-Campylobacter consortium (IP7)

*Keywords: Campylobacteriosis, vitamin C, ascorbate, intestinal and extra-intestinal including systemic anti-inflammatory effects*

Background and objectives: The health-beneficial, particularly anti-inflammatory effects of ascorbate (vitamin C) are well known. In the present preclinical intervention study we assessed potential anti-pathogenic and immunomodulatory effects of ascorbate in a murine acute campylobacteriosis model.
Materials and methods: Secondary abiotic IL-$10^{-/-}$ mice were subjected to ascorbate treatment via the drinking water starting four days before peroral C. jejuni 81-176 strain challenge.
Results: At day 6 postinfection, ascorbate-treated mice harbored slightly lower colonic pathogen loads and suffered from less severe C. jejuni-induced enterocolitis as compared to placebo controls. Ascorbate treatment did not only alleviate macroscopic sequelae of infection, but also dampened apoptotic cell and pro-inflammatory immune responses in the intestines, whereas cell regenerative measures were promoted. Remarkably, the anti-inflammatory effects of ascorbate treatment in C. jejuni infected mice were not restricted to the intestinal tract, but could also be observed in extra-intestinal compartments including liver, kidneys and lungs.
Conclusion: Ascorbate constitutes a promising compound exerting potent anti-inflammatory effects during acute campylobacteriosis.

## Characteristics of a P1-/P7-like prophage mediating transmission of an incorporated transposon comprising a $bla_{CTX-M-15}$ resistance gene

Jens Andre Hammerl, Claudia Jäckel, Valerie Osieka, Silvia Schmoger, Claudia Szentiks, Annemarie Käsbohrer

Federal Institue for Risk Assessment (BfR), Berlin, Germany

*Keywords: phage, whole genome sequence (WGS), antimicrobial resistance, ESBL, plasmid-prophage*

To characterize the genetic basis of ESBL-producing E. coli and its mechanisms for the transmission of the cephalosporin resistance, isolates recovered from wildlife in Germany between 2015 and 2017 were further characterized. In this study, an E. coli was identified harboring a P1-/P7-like prophage exhibiting $bla_{CTX-M-15}$ resistance determinant.
S1-PFGE profiling, transformation, resistance testing and lysogenization was performed to characterize the properties of the isolate and its mobile genetic elements. Mitomycin C-inductions were conducted to assess the activity of the prophage.
Genome determination of the E. coli revealed a $bla_{CTX-M-15}$ carrying sequence contig exhibiting significant homologies to known P1-/P7-plasmid prophages. Plaque tests indicated that the phage possess a broad host range. The phage showed typical myoviral morphology and infects various E. coli of different serotypes. Due to the incorporation of the phage, lysogenic conversion of the recipient bacteria was observed by the production of extended-spectrum beta-lactamases.
P1-/P7-like plasmid prophages might be efficient vehicles for the transfer of antimicrobial resistance determinants. As the resistance genes are often associated with transposon sequences the dissemination of the resistances is further forced by their activity and specificity. The transfer of antimicrobial resistances by phages may represent an evolutionary adaption to extend the number of possible intra- and interspecies hosts.

## Impact of two antibiotic regimes on composition and diversity of gut microbiomes in horses with colic surgery – a comparative analysis

Anne Kauter[1], Antina Lübke-Becker[2], Dania Kanapin[3], Sabita Diana Stöckle[3], Lennard Epping[4], Torsten Semmler[4], Lothar H. Wieler[5], Heidrun Gehlen[3], Birgit Walther[1]

[1]Robert Koch Institute ZBS4 Advanced Light and Electron Microscopy, Berlin Germany
[2]Institute of Microbiology and Epizootics, Berlin Germany
[3]Equine Clinic: Surgery and Radiology, Berlin Germany
[4]Robert Koch Institute NG1 Microbial Genomics, Berlin Germany
[5]Robert Koch Institute, Berlin Germany

*Keywords: equine microbiome, multidrug resistance, shannon diversity*

Recent studies identified veterinary clinics as hot-spots for spread of multidrug-resistant (MDR) bacteria, including zoonotic indicator pathogens (IP) such as MDR-Enterobacteriaceae and Acinetobacter baumannii. The aim of this study is to promote novel perioperative antibiotic prophylaxis strategies against further accumulation of MDR bacteria in clinics providing healthcare for horses.
Here, horses subjected to colic surgery receive either a "single shot" antibiotic course prior surgery or a five day-lasting course, in both cases a combination of gentamicin/penicillin. Fecal samples are collected directly at hospital admission (t0) as well as on day three (t1) and 10 (t2) after surgery. All samples are divided and screened for MDR-IP by conventional microbial diagnostics and subjected to 16S rRNA extraction and sequencing.
Preliminary results are available for 56 horses included so far. Overall, 7% of the fecal samples from t0 were found positive for MDR-E. coli and 7% for A. baumannii-complex. Analysis of the first 16S rRNA data sets (ten horses) revealed changes in both, gut microbiome composition and -diversity. Shannon diversity indices indicated that the "single shot" antibiotic course seems to allow an earlier recovery from loss of microbiome diversity (t1 à t2: 5,1 vs. 6,4). Increasing the sample size and analysis of additional shotgut metagenomics data together with the conventional diagnostic results is required to understand antibiotic driven changes in the equine microbiome.

## Bacterial adaptation to surfactant antimicrobials

O. Makarova[1], P. Ferguson[2], P. Johnston[3,4,5], A. J. Mason[2], J. Rolff[3], U. Roesler[1]

[1]Institute of Animal Hygiene and Environmental Health, Centre for Infection Medicine, Freie Universität Berlin, Berlin, Germany;
[2]Institute of Pharmaceutical Science, King's College London, UK;
[3]Evolutionary Biology, Institute for Biology, Freie Universität Berlin, Berlin, Germany;
[4]Berlin Center for Genomics in Biodiversity Research, Berlin, Germany;
[5]Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany

*Keywords: biocide, antimicrobial peptide, resistance*

Background and objectives: Cationic surfactants are potent antimicrobials and include benzalkonium chloride (BAC), a common disinfectant, and pexiganan (PEX), a therapeutic antimicrobial peptide. Both BACs and PEX act on bacterial membranes. But do these similarities lead to the similarities in resistance mechanisms and cross-resistance, compromising their clinical use?
Materials and methods: Stable resistant mutants of Staphylococcus aureus were generated by passaging daily in increasing concentrations of BAC or PEX, and analysed by whole genome sequencing and metabolomics. Minimum inhibitory concentrations were determined for BAC, PEX and a panel of antibiotics. Fitness costs were assessed by growth curves.
Results: Adaptation to PEX was fast and high-level (32-fold, five transfers) compared to BAC (4-fold, seven transfers). Mutations associated with phospholipid metabolism and efflux pump activity were found in PEX and BAC mutants, respectively. Metabolomics confirmed vastly different cellular responses. There were no changes in cross-resistance to BAC, PEX and antibiotics. PEX mutants had high fitness costs, while BAC mutants showed none.
Conclusion: We find no support that concurrent use of PEX and BAC results in mutual cross-resistance and resistance to antibiotics in S. aureus due to a shared mechanism of action. While low level of resistance evolution recommends BAC as an efficient biocide against Staphylococci, absence of fitness costs in resistant mutants is worrying.

## The mode of action of *T. gondii* tissue cyst inhibitors

Deborah Maus, Jens Pikkemaat, Elyzana Putrianti, Martin Blume

Robert Koch-Institute, Junior Group 2, Berlin, Germany

*Keywords: Toxoplasma gondii, metabolomics, mode of action, inhibitors*

The intracellular, apicomplexan parasite Toxoplasma gondii infects up to 30% of the global human population and causes life-threatening diseases in immuno-compromised patients. Chronically persisting bradyzoites form cysts in brain and muscle tissues and are responsible for transmission and remission of this disease. However, currently available medical treatment options are only effective against the virulent tachyzoites but fail to target the chronic stages of T. gondii.

To address this shortcoming, we are screening various antimicrobial compound libraries against both stages of the parasite in a plate based assay by observing a growth-dependent fluorescence. Potentially effective substances have an unknown molecular target in T. gondii, which we want to identify with an untargeted metabolomics approach using HILIC-UHPLC-MS: The global metabolic response of the parasite to candidate drugs will be compared to established inhibitors with known modes of action. In subsequent reverse-genetic, knock-down und fluxomics experiments we will further characterize the potential T. gondii cyst inhibitor and its molecular target.

## Antimicrobial usage in German cattle farms - which groups of active ingredients are used?

Katharina Hommerich[1], Maria Hartmann[2], Svetlana Kasabova[2], Annemarie Käsbohrer[3], Lothar Kreienbrock[2]

[1] Department of Biometry, Epidemiology and Information Processing University of Veterinary Medicine Hannover
[2] Federal Institute for Risk Assesment

*Keywords: antimicrobial consumption, critically important antimicrobials, antimicrobial resistance, dairy cattle, beef cattle*

The VetCAb study (Veterinary Consumption of Antibiotics) is a longitudinal study with ongoing participant recruitment to describe antimicrobial usage in livestock in Germany. Data collection is based on official application and delivery forms, voluntarily provided by veterinarians and farmers. We analyzed the half-year treatment frequency (TF) and the percentage of active substances used for treatment of dairy and beef cattle on farm level for the years 2013 to 2017. A particular focus was set on the "Highest Priority Critically Important Antimicrobials" (HPCIA) defined by the World Health Organization, which are of essential importance for the treatment of specific infections in humans.
The calculated TF on dairy farms varied at a constantly low level between 1.9 and 2.4 for dairy cows and 0.3 and 0.9 days for calves over the entire period. Regarding the use of HPCIAs in dairy cows, fluorquinolones played a major role with around 15% of the total TF mainly used for treating diseases of the reproductive organs. In calves, aminoglycosides and penicillins made up the largest shares of the TF (40%), respectively. The majority of the few treatments in beef cattle (TF=0) were made up of tetracyclines and trimetoprim/sulfonamide combinations.
Our results provide knowledge about the antibiotic substance classes used in Germany in connection with production group, indication and administration route and support the evaluation of antimicrobial resistance development in cattle farming.

## Plenary Session III: Keynotes

**October 18, 2019**
**11:30 – 15:00**

**Room Ballsaal**
**Chair: Stephan Ludwig**

## Human filarial infections – a group of NTDs with a zoonotic relative of increasing importance

Achim Hoerauf[1]

[1]Bonn University Medical Center, Germany

*Keywords: filariasis, onchocerciasis, dirofilariasis, drug development, NTD*

Among the 20 infections and diseases listed by the WHO as Neglected Tropical Diseases (NTDs), all of them except snakebite are infectious. The ones that have been targeted for elimination usually are those that are (mostly) *not* a zoonosis, such as human filarial infections onchocerciasis and Lymphatic filariasis (plus others not listed as NTDs). Over the last decade, a lot of progress has been made towards elimination of these infections, however it has become clear that without new drugs the ambitious SDG goals will not be reached. In my talk I will review recent developments in drug research against human filarial nematodes that are in or past clinical development and probably will make an impact over the next years. Some of the new drugs originate from the veterinary market, targeting Dirofilariasis, a very widespread infection of cats and dogs worldwide in warm climates. Vice versa, treatment of Dirofilaria with tetracyclines, now present in many official recommendations, has been conceptually aided by successful protocols of treatment of human filariasis with doxycycline, which targets Wolbachia endosymbionts. Dirofilariasis is a classical zoonosis, erroneously infecting humans, thereby imposing as tumors and often leading to lengthy and stressing exclusion diagnosis. This could be avoided if dirofilariasis, through climate change an emerging infection of humans, were better kept in the differential diagnosis loop.

## Nipah Virus: An Emerging Zoonosis and Global Health Threat

Jonathan H. Epstein[1]

[1]EcoHealth Alliance, New York, USA

*Keywords: Emerging zoonoses; bats; Nipah virus; Pteropus medius; spillover*

Nipah virus is an emerging, highly pathogenic zoonotic virus and select agent capable of causing public health emergencies of international concern. In 2018 the WHO listed Nipah virus as one of the top ten threats to global health and security. Nipah virus was first recognized in Malaysia in 1998, and has subsequently caused recurring outbreaks in India and Bangladesh, and a single outbreak in the Philippines. In Bangladesh, Nipah virus outbreaks have been reported almost annually since 2001. Though typically limited in scale, human-to-human transmission has been a common feature of Nipah virus outbreaks. In Bangladesh, people are exposed to Nipah virus via the consumption of date palm sap which has been contaminated by bat excreta. *Pteropus medius,* a common giant frugivorous bat, has been identified as the primary reservoir for Nipah virus in Bangladesh and has also been shown to carry Nipah virus in India. In May 2018, an outbreak was reported in Kerala, India – the first in southern India and the first reported case in India since 2007 in West Bengal. In Kerala, there were 23 cases reported, 21 which were fatal. In June 2019, a new case was diagnosed in Kerala, however, no additional cases were involved. In both instances, the initial route of spillover from bat to the index case was not determined, although local *Pteropus giganteus* sampled during the outbreak investigation tested positive for Nipah virus RNA. Hospital-based transmission was the main cause of secondary cases in 2018, which included health care workers. The outbreaks in Kerala and prior findings in northern India and Bangladesh suggest that Nipah virus circulates in *P. medius* throughout the region and that spillover is possible wherever these bats and people co-occur. This presentation will review Nipah virus ecology, epidemiology and discuss gaps in knowledge that need to be filled in order to understand the risk of future Nipah virus outbreaks.

## Poster Presentations

## Room: Lankwitz, Foyer, Atrium

## Schedule

| | | |
|---|---|---|
| **Wednesday, October 16th** | **18:50** | **all posters** |
| **Thursday, October 17th** | **10:30 – 11:00** | **even poster numbers** |
| | **12:30 – 13:30** | **even poster numbers** |
| | **13:30 – 14:30** | **odd poster numbers** |
| | **19:00 – 20:00** | **odd poster numbers** |
| **Friday, October 18th** | **11:00 – 11:30** | **all posters** |
| | **12:15 – 13:15** | **all posters** |

## Room Allocation

| Room | Poster numbers | General topic |
|---|---|---|
| **Atrium** | **C01 – C21** | **Pathogen-Cell Interactions** |
| | **P01 – P09** | **Parasitic Zoonoses** |
| **Lankwitz** | **A01 – A27** | **Antimicrobial use and resistance** |
| | **M01 – M09** | **Novel Methods** |
| **Foyer** | **D01 – D10** | **Diagnosis** |
| | **E01 – E22** | **Epidemiology and Ecology** |
| | **I01 – I10** | **Innate and Adaptive Immune Response** |
| | **N01 – N31** | **New and re-emerging Zoonoses** |
| | **S01 – S04** | **NGS** |
| | **H01 – H17** | **Public Health** |

# Poster Presentations Innate and Adaptive Immune Response

## I01 – I10

# I01

## Innate immunity phenotype of viruses

Ulrike Felgenhauer, Nadja Karl, John Ziebuhr, Friedemann Weber

JLU Giessen

*Keywords: antiviral; innate immunity; interferon; interferon-stimulated gene*

Interferons are the first line of the innate immunity, triggering the expression of a large number of interferon stimulated genes (ISGs) of which several are antiviral. Pathogenic viruses are able to counteract the induction of interferon and block interferon signaling. The quality and strength of interferon evasion and ISG sensitivity (namely, the innate immunity phenotype) is an important marker of virulence.
We aim to characterize the innate immunity phenotype of newly emerging viruses. We seek to generate a virus profile that differentiates between low pathogenic and highly pathogenic viruses. Comparing the behaviour of a newly emerging virus to the data set we are in the process of creating, may serve as a tool for the rapid risk assessment of emerging viruses.
First, we are characterizing the interferon sensitivity of several coronaviruses and influenza viruses by quantifying virus production after pretreatment with varying amounts of interferon. Second, we are generating a set of stable cell lines that inducibly overexpress select ISGs, in order to later quantify virus growth/cell survival on these cell lines.
This categorization of viruses with respect to their innate immunity phenotype may in the future give a first estimate of the risk associated with newly emerging zoonotic viruses.

# I02

## Vitamin D and acute campylobacteriosis – results from an intervention study applying a clinical Campylobacter jejuni induced enterocolitis model

Soraya Mousavi, Ulrike Escher, Anna-Maria Schmidt, Stefan Bereswill, Markus M. Heimesaat

Institute of Microbiology & Infection Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany; PAC-Campylobacter consortium (IP7)

*Keywords: Campylobacteriosis, vitamin D, 25-hydroxy-cholecalciferol, intestinal and extra-intestinal including systemic anti-inflammatory effects*

Background and objectives: In the present preclinical intervention study we investigated anti-pathogenic and immuno-modulatory properties of vitamin D applying an acute campylobacteriosis model.

Materials and methods: Secondary abiotic IL-10-/- mice were perorally treated with synthetic 24-OH-cholecalciferol via the drinking water starting four days before peroral Campylobacter jejuni infection.

Results: Whereas vitamin D application did not affect gastrointestinal pathogen loads, vitamin D-treated mice suffered less frequently from diarrhea in the midst of infection as compared to placebo controls. Moreover, vitamin D application dampened C. jejuni-induced apoptotic cell responses in colonic epithelia and promoted cell-regenerative measures. At day 6 post-infection, vitamin D treated mice displayed lower numbers of colonic immune cell populations as compared to placebo controls that were accompanied by lower large intestinal concentrations of pro-inflammatory mediators. Remarkably, vitamin D-treatment of C. jejuni-infected mice resulted in less frequent translocation of viable pathogens from the inflamed intestines to extra-intestinal including systemic compartments such as the kidneys and spleen, respectively.

Conclusion: Our preclinical intervention study provides evidence that peroral vitamin D application exerts inflammation-dampening effects during acute campylobacteriosis.

# I03

## Development and validation of a species-independent whole proteome tick-borne encephalitis virus antibody detection assay

Laura Wiesner[1,2], Mathias Boelke[1,3], Claudia Schulz[1,3], Natascha Spitmann[4], Reinhard Mischke[4], Christine Bächlein[5], Paul Becher[5], Stefanie Becker[1,3] and Imke Steffen[1,2]

[1]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover, Germany; [2]Institute for Biochemistry, University of Veterinary Medicine, Hannover, Germany
[3]Institute for Parasitology, University of Veterinary Medicine, Hannover, Germany
[4]Small Animal Clinic, University of Veterinary Medicine, Hannover, Germany
[5]Institute of Virology, University of Veterinary Medicine, Hannover, Germany

*Keywords: tick-borne encephalitis virus, flavivirus, luciferase immunoprecipitation system, serology, antibody*

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis, the most common infection of the central nervous system in endemic regions of Europe and Asia. Besides humans, various animal species are susceptible to TBEV infection with clinical manifestations ranging from asymptomatic to severe neurological complications like meningitis or meningoencephalitis. To compare the protective role of anti-TBEV antibody responses in different hosts and between individuals, we developed a luciferase immunoprecipitation system (LIPS) antibody detection assay. All ten TBEV proteins were expressed as fusion proteins with nano-luciferase and used for the detection of specific antibodies in serum from different hosts. Expression of all antigen fusion proteins was confirmed and acceptable assay performance was verified with intra- and inter-assay coefficients of variation of 21% and 17%, respectively. Sera from different species, such as deer, boar and dogs were screened for antibodies against all TBEV antigens. The most abundant antibodies targeted the E and NS1 proteins, but minor antibody populations against other antigens were detected in some samples. Testing of 399 dog sera by ELISA and LIPS assay found four reactive samples, resulting in a prevalence rate of 1%. The LIPS assay exhibited high sensitivity and correlated well with ELISA and neutralization data. Whole proteome antibody profiles will help identify protective and cross-reactive flavivirus epitopes.

# I04

## Unravelling mechanisms of rabies virus-induced immunosuppression

Embregts, C.W.E., Kuiken, T., Koopmans, M., GeurtsvanKessel, C.

Department of Viroscience, Erasmus Medical Centre, Rotterdam, the Netherlands

*Keywords: rabies virus, innate immunity, immunosuppression*

Rabies encephalitis is caused by infection with a lyssavirus, including rabies virus (RABV), and is transmitted through a bite or a scratch of an infected animal. RABV uses multiple ways to evade and suppress the immune system and is able to migrate to the brain unnoticed; no evident local immune response is observed at the bite/scratch site and no specific antibody titres are mounted. Given that the understanding of the (early) evasion and suppression of the immune response is of utmost importance to develop novel treatment strategies against the disease, we investigated the effect of RABV on various immune cells in order to identify mechanisms involved in RABV-induced immunosuppression.
Various cell types, including antigen presenting cells and lymphocytes, were isolated from blood of healthy human volunteers and were exposed to RABV in vitro. The response of these cells to RABV was measured using multiple assays with a focus on inhibition of the inflammatory response. To study the downstream effects of locally affected cells on the lack of a specific immune response, various co-cultures (e.g. macrophage and T cell) were established.
We show that RABV directly binds to macrophages, an important infiltrating cell type at the bite site, which triggers multiple immune pathways resulting in dampening of the immune response.

# I05

## Immune responses to Lassa virus infections in mice and humans

David Wozniak[1], Lisa Oestereich[2], Julia Port[2], Elisa Pallasch[2], Sabrina Bockholt[2], César Muñoz-Fontela[2], Stephan Günther[2]

[1]Robert Koch-Institut
[2]Bernhard-Nocht-Institute for Tropical Medicine

*Keywords: Lassa virus, acute infection, small animal model, human infection*

Lassa fever is a highly pathogenic viral syndrome endemic to West Africa and often resulting in death. Despite ongoing research since its discovery in 1969, the pathophysiology of Lassa virus (LASV) infections is largely unknown. The natural reservoir host of LASV is the rodent Mastomys natalensis, in which the infection is non-symptomatic. One constrain of basic research on LASV, besides it BSL4-status, has been the lack of small animal models that reproduce symptoms of the human disease. Immunocompetent laboratory mice are resistant to an infection with LASV. Oestereich et al. established a susceptible, highly lethal mouse model with a wild-type hematopoetic immune response. These IFNAR$^{-/-}$ bone marrow-chimeric mice with bone marrow from wild-type mice closely mimic the symptoms found in the human disease of Lassa fever, including liver damage, vascular leakage, systemic virus dissemination and death.
Further, flow cytometric immunological data acquired onsite at the Irrua Specialist Teaching Hospital in Nigeria during annually occurring Lassa fever outbreaks enabled comparative studies of the disease in humans with the established mouse model. Multiplex cytokine analyses of serum revealed similar inflammatory cytokine responses between human Lassa fever cases and the mouse model, while a strong CD8$^{+}$ T cell activation seen in the mouse model could not be observed in humans. Comparative analyses will continue to shed light onto the immune responses during LASV infection.

# I06

## Correlates of protection against tick-borne encephalitis in BL/6 mice provided by pre-infection with Langat virus

Monique Petry[1], JG Mitterreiter[1], M Palus[2], S Schreier[3], A Kröger[3], GF Rimmelzwaan[1], GMGM Verjans[4], D Ruzek[2], ADME Osterhaus[1]

[1] Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover, Germany
[2] Department of Virology, Veterinary Research Institute, Brno, Czech Republic
[3] Innate Immunity and Infection, Helmholtz Centre for Infection Research, Braunschweig, Germany
[4] Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands

*Keywords: Tick-borne encephalitis virus, Langat virus, antibody and T-cell immunity, adoptive transfer, correlate of protection*

Tick-borne encephalitis virus (TBE) is the most important arthropode-transmitted viral disease in Europe with continuously growing endemic areas. It is caused by TBE virus (TBEV), a positive single-stranded RNA (11kb) virus belonging to the Flaviviridae family. TBEV is closely related to Langat virus (LGTV), a naturally attenuated serogroup member of TBEV (82-88% amino acid identity), that has been considered as a vaccine candidate. The current vaccine does not guarantee sufficient protection with boosters needed every 3-5 years. Therefore, this project investigates the host immune response to LGTV infection that protects against TBEV infection.

A first mouse vaccination experiment was performed to evaluate the protective effect of LGTV infection against TBE. Therefore, mice were infected with LGTV and 28 days post infection (dpi) challenged with TBEV Hypr or TBEV 280 (attenuated strain). Results showed a strong vaccination effect of LGTV infection. All LGTV 'vaccinated' mice survived, while all unvaccinated mice died or had to be sacrificed 8-10 dpi.

Subsequently, an adoptive transfer study was performed to determine correlates of protection. Donor mice were infected with LGTV. 28 dpi, naïve recipient mice received serum, $CD4^+$ T-cells, $CD8^+$ T-cells or $CD3^+$ T-cells from the LGTV preinfected animals. 24 hours later they were infected with TBEV Hypr. All mice with serum transfer survived without symptoms. The $CD4^+$-T-cell transfer showed significantly prolonged survival.

# I07

## Investigating the antiviral effect of the RNAi pathway on arbovirus infection in *Aedes aegypti*

M. Leggewie[1,2], M. Badusche[1], E. Schnettler[1,2]

[1]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.
[2]German Centre for Infection research, partner side Hamburg-Lübeck-Borstel Riems, Germany

*Keywords: RNAi, SFV, Aedes aegypti*

RNA interference (RNAi) is considered to be the cornerstone of antiviral immunity in insects. Current work strongly indicates that especially the small interfering (si)RNA and the Piwi-interacting (pi)RNA pathways are initiated during viral infections in mosquitoes. A lot of in vitro studies focusing on arboviruses, from different virus families, have been performed. But little is known about the situation in vivo. Understanding the interactions between viruses and the mosquito immune system is beneficial for understanding the development and dynamics of vector competence.

Here, we used the Semliki Forest alphavirus (SFV) as a model virus to set up an in vivo-knock down assay in adult Aedes aegypti. dsRNA targeting key proteins of the piRNA and siRNA pathways are injected into the mosquitoes. Upon establishing the knock down phenotype, the mosquitoes are infected with SFV via a blood meal and changes in virus replications are monitored.

In vivo knock-downs in Ae. aegypti have been established for Ago2. Interestingly, although the infection rate is not affected by Ago2-knock down at 3 days post infection, the SFV titer was found to be significantly increased in comparison to control individuals.

These data give a first indication, that the siRNA pathway acts antiviral against SFV within an in vivo system. This system will be used to investigate the effect of RNAi on the infection process of other arboviruses and estimate its implications on vector competence.

# I08

## Rousettus Bat Myeloid Cells Respond to Marburg virus Infection by Upregulation of Interferon-related Genes and Downregulation of Pro-inflammatory Mediators

Joseph Prescott[1,2,*], Jonathan C. Guito[2], Jessica R. Spengler[2], Catherine E. Arnold[3], Jonathan S. Towner[2]

[1]Center for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany
[2]Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA 30329, USA
[3]Diagnostic Systems Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702, USA

Infection with hemorrhagic fever-causing zoonotic viruses leads to dysregulation of the immune system and subsequent disease in humans. Elucidating mechanisms of how the natural animal reservoirs of these viruses are able to co-exist with these agents without overt disease, while permitting sufficient replication for transmission and maintenance in a population, is important for understanding the ecology of these viruses and the human disease. The Egyptian rousette bat has been identified as a reservoir for Marburg virus (MARV), a filovirus and the etiologic agent of the highly lethal Marburg virus disease. Little is known regarding how these bats immunologically respond to MARV infection. In humans, macrophages and dendritic cells (DCs) are primary targets of infection and their dysregulation is thought to play a central role in filovirus diseases, by disturbing their normal functions as innate sensors and adaptive immune response facilitators, while serving as amplification and dissemination agents for the virus. The infection status and responses to MARV in bat myeloid-lineage cells is uncharacterized, and likely represents an important modulator of the bat's immune response to MARV infection. Herein, we generate dendritic cells (DCs) from the bone marrow of rousette bats. MARV infection resulted in a low level of transcription in these cells and significantly downregulated DC maturation and adaptive immune stimulatory pathways, while at the same time upregulated interferon-related pathogen sensing pathways. This study provides a first insight into how the immune response in rousettus bats is directed towards preventing aberrant inflammatory responses, while mounting an antiviral response toward MARV infection.

# I09

## Vaccine-induced immune responses in Gabonese infants

Judith Flügge[1,2], Josiane Honkpehedji[3], Esther Askani[1,3], Marguerite M. Loembe[1,3,4], Thaisa L. Sandri[1], Sina Brückner[1], Mohammed Duali[3], Johannes Strunk[1], Benjamin Mordmüller[1,2,3], Selidji T. Agnandji[1,3], Bertrand Lell[1,2,3], Peter G. Kremsner[1,2,3], Ayôla A. Adegnika[1,2,3], Meral Esen[1, 2, 3]

[1]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany
[2]Deutsches Zentrum für Infektionsforschung, Tübingen, Germany
[3]Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, [4]Université des Sciences de la Santé, Libreville, Gabon

*Keywords: parasite infection, helminths, vaccine immunogenicity*

Vaccine-preventable diseases, such as measles, pertussis, tetanus and poliomyelitis are still responsible for high disease burden worldwide. The impaired protection occurs mainly in low- and middle-income countries and seems therefore to be associated with incomplete coverage, malnutrition and bacterial infections. Helminths are known to modulate immune response of their host towards Th2, which can affect immune response towards vaccines. Even prenatal exposure to parasitic antigens has been shown to impact the development of the neonate´s immune system but this is not well studied so far.
To assess the impact of maternal helminth infections during pregnancy on the humoral vaccine immunogenicity of the offspring, we conducted a study in Lambaréné and surroundings, Gabon. Pregnant women in their last trimester were screened for helminths and assigned to groups based on their helminth status. Infection was diagnosed microscopically by the Kato-Katz, copro-culture, urine filtration and saponin haemolysis methods. After birth, infants received the vaccines given within the Expanded Program on Immunization (EPI) and were followed-up until 1 year of age. Antibody titers to measles, diphtheria, pertussis, tetanus, poliomyelitis, hepatitis B and Haemophilus influenza type B (HiB) vaccines were measured from mother and cord blood at delivery and from children at 9 and 12 months of age using commercial and validated ELISA Kits.
A total of 123 mother-child pairs were available for analysis. 42.3 % of the mothers were infected with helminths, Schistosoma haematobium being the most prevalent.
Despite a trend towards lower immune responses in children born to helminth-infected mothers, we found no significant difference in vaccine related IgG in infants from helminth-infected versus non-infected mothers within the investigated timepoints.
Our data show that infection with helminths is still common in pregnant women in Gabon but has only subtle, presumably no clinically significant effects on infant's immune response to vaccines given as part of the EPI.

# I10

## Toll-like receptor 9 is essential for innate immunity during neonatal listeriosis

Dennis Pägelow[1], Melanie Brinkmann[2], Mathias Hornef[3] & Marcus Fulde[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin;
[2]Institute of Genetics – Biozentrum, Technische Universität Braunschweig, Braunschweig;
[3]Institute for Medical Microbiology, University Hospital RWTH Aachen, Aachen, Germany

*Keywords: Listeria monocytogenes, neonatal innate immunity, neonatal murine infection model*

Background and objectives: Early recognition by the innate immune system is key for successful defense against Listeria monocytogenes (Lm). Although Lm still represents an important cause of morbidity in the newborn, the precise roles of Toll-like receptors (TLRs) during neonatal listeriosis remains up to date completely unknown.
Materials and methods: Neonatal C57BL/6 WT as well as TLR9-deficient ($TLR9^{-/-}$) mice were infected intranasally with Lm. To determine bacterial loads, pups were sacrificed at one and three days post infection (dpi) and organs were obtained for replica plating. Tissue tropism and immune responses were analyzed by immunohistochemistry and qRT-PCR.
Results: We found that bacterial loads in nose, brain, lung, liver and spleen were comparable in neonatal WT and $TLR9^{-/-}$ mice at one dpi. At three dpi, CFU counts in $TLR9^{-/-}$ mice were significantly elevated in all investigated organs and the blood. Additionally, mortality rates of $TLR9^{-/-}$ neonates were strongly increased. As demonstrated by histopathological examination, $TLR9^{-/-}$ pups revealed enlarged and necrotic infection foci in nasal, brain and spleen tissue. Finally, qRT-PCR analysis showed an increase of various pro-inflammatory as well as neutrophil- and monocyte-attracting cytokine mRNA expression in brain and spleen tissue of $TLR9^{-/-}$ mice.
Conclusion: We demonstrate for the first time an essential role of TLR9 for innate immunity during systemic and neuronal listeriosis in the newborn mouse after nasal challenge.

# Poster Presentations Publlic Health

## H01 – H17

# H01

## The entry of Listeria monocytogenes into the food chain via slaughter pigs

Verena Oswaldi, Janine Dzierzon, Susann Thieme, Roswitha Merle, Diana Meemken

FU Berlin, Institut für Lebensmittelsicherheit & -hygiene

*Keywords: Listeria monocytogenes, slaughter pigs, foodborne pathogen, listeriosis, asymptomatic carrier*

Listeria monocytogenes is an important human foodborne pathogen and the causative agent of the rare but severe human listeriosis. Listeria species are ubiquitous in the environment and frequently found in raw foods (Allerberger and Huhulescu 2015). Many animals, including pigs, and humans can carry the bacterium without showing clinical symptoms.
To examine if slaughter pigs are a primary source of L. monocytogenes in pork, this study investigates the occurrence of the bacterium in slaughter pigs in northwestern Germany as well as in the slaughtering and processing environment. Fecal and tonsillar samples from 200 fattening pigs from 20 herds immediately after slaughter and environmental samples from the slaughterhouse were qualitatively tested for Listeria spp.
We found a very low detection frequency of L. monocytogenes in tonsils (1%, 2/200) and could not isolate any L. monocytogenes in fecal samples (0%, 0/200). Positive results of L. monocytogenes were found in environmental samples (8%, 6/77).
Due to these results, we consider tonsils of slaughter pigs as a reservoir for L. monocytogenes and as a low but existent risk for contamination of meat products. In order to determine the zoonotic potential of the isolates, the ANSES institute, EU reference laboratory for L. monocytogenes, will subtype the identified Listeria spp. isolates.
This project is funded by the QS Science Fund.

# H02

## Zoonotic brucellosis in Pakistan

Tariq Jamil[1,2], Falk Melzer[1], Muhammad Zubair Shabbir[3], Arfan Ahmad[3], Iahtasham Khan[4], Heinrich Neubauer[1], Stefan Schwarz[2]

[1]Friederich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany
[2]Institue of Microbiology and Epizootics, Free University of Berlin, Berlin, Germany
[3]University of Veterinary and Animal Sciences, Lahore. Pakistan
[4]Section of Epidemiology and Public Health, College of Veterinary and Animal Sciences, Jhang, Pakistan

*Key words: Brucellosis, zoonoses, serology, PCR, Pakistan*

**Background:** Brucellosis is a worldwide zoonotic infection and is an established professional health hazard. Close human-animal relationship and consumption of contaminated unpasteurized milk risks its transmission.
**Methodology:** An outbreak of abortions was reported at a private dairy farm at Jhang, Pakistan. A total of 15/48 pregnant Holstein cows had aborted in their last trimesters. Serum, blood and milk samples were collected for detection of anti-*Brucella* antibodies and *Brucella* DNA by serology, molecular biology and culture examination. Few days later one of the workers at the farm complained about chronic pain in joints muscular pain. Blood and serum samples were also collected from this person.
**Results:** A total of 100% (15/15) aborted animals were found positive by RBPT, iELISA and MRT. The human sample was found seropositive by RBPT and iELISA. None of the samples showed positive by PCR (B4/B5) and culture examination. The animals were treated with Streptomycin and Oxytetracycline and culled later on. The infected person was referred to hospital to seek medical treatment.
**Conclusion:** Brucellosis is a persistent infection in humans and animals. Pasteurization of milk and personal protection while handling animals is recommended. Routine screening for brucellosis is necessary. One health perspective can help fulfilling these objectives. Thus One Health awareness programs should be propagated among farmer population of the country.

# H03

## Serological tests on fattening pigs as a tool for the risk assessment of Hepatitis E virus entering the food chain?

Janine Dzierzon[1], Verena Oswaldi[1], Roswitha Merle[2], Nina Langkabel[1], Diana Meemken[1]

[1]Freie Universitaet Berlin, Institute of Food Safety and Food Hygiene, Section Meat Hygiene
[2]Freie Universitaet Berlin, Institute for Veterinary Epidemiology and Biostatistics

*Keywords: Hepatitis E, fattening pigs, food safety*

With a seroprevalence of up to 96% Hepatitis E virus (HEV) shows a wide distribution among fattening pigs in Germany. The consumption of raw or undercooked pork products represent a potential risk for HEV infection in humans.
Therefore, the objective of this study was to estimate the risk of HEV entering the food chain via pork products based on serological tests comparing with the detection rate of HEV RNA in pork liver and muscle samples from the same pig.
A total of 250 fattening pigs from 25 farms were sampled in an abattoir in North-West Germany.
Per pig, one sample of ham muscle and one sample of liver tissue were collected in order to test these tissues for the presence of HEV RNA by real-time RT-PCR. A sample of the muscle of the diaphragm pillar from the same pig was collected to gain meat juice and to determine the HEV seroprevalence.
In total, 62% (155/250) of the meat juice samples were positive for antibodies against HEV at a single animal basis. So far, HEV RNA could be detected in 14% (18/126) of the liver samples, which came from HEV seropositive pigs.
These results may indicate the possibility of serological tests to assess the risk of the occurrence of HEV RNA in the liver of fattening pigs and to preselect meat and liver from animals from seronegative herds for sensitive meat products.

# H04

## Oral vaccination of dogs; the missing tool to eradicate rabies

Katharina Bobe, Steffen Ortmann, Antje Kretzschmar, Peter Schuster, Ad Vos, Christian Kaiser

IDT Biologika GmbH

*Keywords: rabies, OVD, dog, vaccine*

An estimated 59.000 human cases of rabies occur annually; nearly all cases are dog-mediated. In many countries with dog-mediated rabies, a large proportion of the dog population is not accessible for vaccination by parenteral route.
Oral Vaccination of Dogs "OVD" concept increases efficiency of campaigns by reducing time and costs required to capture and restrain dogs. OVD as a complementary tool to parenteral vaccination increases herd immunity to levels required to interrupt the transmission cycle.
Clinical trials have demonstrated safety & efficacy of a highly attenuated & efficacious 3rd generation rabies vaccine construct in dogs. The practical applicability of the vaccine/sachet/bait under field conditions has been shown, as well as cost-effectiveness of the approach.
OVD represents a viable strategy to supplement existing parenteral vaccination campaigns in hard-to-reach dog populations. The new adaptable sachet/bait concept developed for dog together with the vaccine strain is suitable for numerous other wildlife reservoir species such as raccoon dog, raccoon, skunk, and mongoose. Noninvasive oral vaccination of dogs against rabies is a true and creative One Health approach and is a tool for sustainable success to end dog-mediated human deaths by 2030 launched by OIE, WHO, FAO & GARC.

# H05

## Project ZooM - Zoonotic importance of multidrug-resistant pathogens: FAQs at the interface veterinary/human medicine

Susanne Kutzora, PD Dr. Robin Köck, Dr. Jörg Fritzemeier, Prof. Dr. Caroline Herr, PD Dr. Stefanie Heinze, Dr. Stefan Hörmansdorfer, Dr. Ulla Kandler, Dr. Ute Teichert, Dr. Nicoletta Wischnewski

Bayerisches Landesamt für Gesundheit

In recent years, the number of infections caused by multidrug-resistant bacteria (MDRB) has increased. MDRB are also increasingly important in the private sector (e.g. companion animals), but this aspect has not been sufficiently addressed in previous recommendations. In addition, many persons (e.g. farmers, veterinarians and their household members) acquire MDRB through contact with farm animals. The guidelines of the Commission for Hospital Hygiene and Infection Prevention (KRINKO) at the Robert Koch-Institute as well as the Technical Rules for Biological Agents (TRBA) in Germany focus on recommendations for healthcare facilities for the prevention of nosocomial or occupational infection risks. There are no recommendations for the private sector or for non-medical facilities and institutions. Consequently, in the public health service, answers regarding many questions related to (zoonotic) MDRB in these sectors are lacking.
The ZooM project deals with identifying open questions regarding the zoonotic significance of MDRB at the interface between veterinary and human medicine. First interview results were already presented at the "retreat" meeting of the Research Network Zoonotic Infectious Diseases in May 2019 with regard to different subordinate aspects: farming, companion animals, therapy animals and wildlife animals.
Preliminary results from several interviews with public health authorities indicate that there are major uncertainties regarding livestock-associated MRSA among persons with occupational exposure as well as how to cope with MDRB in households and kindergartens.
These frequently asked questions (FAQs), which are relevant for both the human and veterinary public health services, will be answered and disseminated within the project. Subsequently, the FAQs and their dissemination will be evaluated with regard to their benefit/usefulness for the daily work of the public health service (human and veterinary medicine).
The ZooM project is funded by the Federal Ministry of Education and Research (BMBF) as part of the Zoonotic Infectious Diseases Research Network.

# H06

## Canine Brucellosis and Brucella canis in Humans: Systematic Review of the Literature

D.A.V. da Silva[1], I. Boone[1], D. Schlichting[1], S. Al Dahouk[1,2]

[1]German Federal Institute for Risk Assessment, Berlin, Germany
[2]RWTH Aachen University Hospital, Germany.

*Keywords: Brucella canis, canine brucellosis, one health, epidemiology*

Brucellosis is a globally disseminated zoonosis. The infection is usually transmitted to humans either by direct contact with infected livestock or through the consumption of derived products. B. canis is the most common cause of brucellosis in dogs, but human infections are rarely reported although dogs are close companion animals. To get a deeper insight into the epidemiology of this potentially neglected type of brucellosis, we performed a systematic review of the literature.
Bibliographic databases were screened using pre-defined search terms. Studies reporting on canine brucellosis or B. canis infections in humans were included in our analysis.
A total of 384 studies published between 1933 and 2019 originating from all continents (51 countries) met our inclusion criteria. Most articles described B. canis infections in dogs, however, other Brucella species were also reported. The diagnosis of canine brucellosis relied on a tremendous variety of serological (n=28) and molecular (n=5) tests conducted in parallel to bacterial isolation via different culture methods. In one third of the studies, transmission of B. canis from dogs to humans could be verified.
Canine brucellosis has been reported from all over the world for almost a century. The number of human cases continuously increased but may be still underestimated because serological tests commonly used for the diagnosis of other types of brucellosis are inappropriate for B. canis infections due to an antigen mismatch.

# H07

## Detection of Puumula Hantaviruses in environmental samples near households as a basis for targeted prevention and infection control

Jenny Krieg, R Oehme[1], J Fritz[2], H Turni[3], M Bauer[4], A Corea[5], C Finkbeiner[6], K Gronbach[7], S Haag-Milz[8], T Hierl[9], N Mürter[10], I Saalmüller[11], R Ulrich[12], M Eichner[13], SO Brockmann[2]

[1]Landesgesundheitsamt Baden-Württemberg
[2]Kreisgesundheitsamt Reutlingen
[3]Stauss & Turni
[4]Gesundheitsamt Zollern-Alb-Kreis
[5]Gesundheitsamt Esslingen
[6]Gesundheitsamt Heidenheim
[7]Gesundheitsamt Stuttgart
[8]Gesundheitsamt Sigmaringen
[9]Gesundheitsamt Tübingen
[10]Gesundheitsamt Göppingen
[11]Gesundheitsamt Böblingen
[12]Friedrich-Löffler Institut
[13]Universität Tübingen

*Keywords: Environment, bank vole, Puumala Hantavirus, infection control and prevention*

Puumala Hantavirus (PUUV) is a zoonosis with growing public health importance; the virus belongs to the list of prioritized zoonotic pathogens in Germany. Preventive measures mostly are not evidence based and their impact is not well assessed. In order to improve the prevention of PUUV disease, a better understanding of risk factors and effectiveness of preventive measures is required. We try to detect PUUV in environmental samples and rodents taken near households in highly endemic areas in order to identify possible exposure sites for human PPUV infection.
In 60 households within eight administrative districts in the State of Baden-Wuerttemberg, environmental samples are retrieved three times per season, over a period of two years. Standardized sampling takes places at defined locations in the domestic environment (i.e. garden shed, terrace, loggia, barn, wood stack, garden tools). Samples are purified by using a silica extraction and analyzed via RT-PCR. PCR-positive samples will be further typed and cultivated.
We will present the results of the first investigation year. After establishing the methods successfully, we already detected PUUV in nine samples of the first three households (9 of 46 samples). Positive samples originate from wood stacks, garden sheds and a terrace.
The findings contribute to a better understanding of PUUV epidemiology and aim to tailor Public Health intervention strategies and recommendations for the prevention of PUUV disease.

# H08

## Fresh chicken meat harbors toxinogenic strains of Clostridium difficile

Heise, J.[1], Witt, P.[1], Maneck, C.[1], Wichmann-Schauer, H.[1], Maurischat, S.[1]

[1]German Federal Institute for Risk Assessment, Unit Microbial Toxins, Department Biological Safety, Berlin

*Keywords: C. difficile, diarrhea, zoonotic transmission, food*

Clostridium (C.) difficile is a well-known pathogen of elder humans and major cause of antibiotic-associated diarrhea. Nowadays, C. difficile infection (CDI) is increasingly registered independently of hospitalization and the age of the patients. One potential cause of the so-called community-acquired infection is a zoonotic transmission to humans based on direct contact with animals or the consumption of food, indicated by closely related isolates from humans and animals as well as the isolation of C. difficile from different food products.
To estimate the exposition of humans with C. difficile via food, we analyzed the occurrence of C. difficile in 311 different retail products of fresh chicken meat. We detected C. difficile in 14.1% (n= 270, with skin) and 0% (n= 41, without skin) of the tested chicken meat samples. Most isolates exhibit toxin genes tcdA and tcdB. The isolates were mainly represented by PCR-ribotypes 001, 002, and 014, which are also frequently detected in human CDI cases in Germany and were partially detected in poultry.
The results of this study reveal that fresh retail chicken meat with skin in Germany is often contaminated with toxinogenic C. difficile. The presence of PCR-ribotypes already detected from CDI patients indicates that contaminated chicken meat may be a potential source of human CDI.

# H09

## *C. difficile* may cause infections in reptiles

D. Rabold[1], L. Mundhenk[2], C. Seyboldt[3], A. Lübke-Becker[1]

[1]Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Germany;
[2]Institute of Veterinary Pathology, Department of Veterinary Medicine, Freie Universität Berlin, Germany;
[3]Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut), Jena, Germany.

*Keywords: C. difficile, reptile, zoonosis*

Clostridioides (previously Clostridium) difficile is a potential pathogen for humans and animals. Human C. difficile infections (CDI) are often healthcare associated with varying gastrointestinal tract symptoms. Nevertheless, one quarter of human CDI is estimated to occur within the community, whereby the source of infection remains unknown. A high overlap of strains occurring in humans and various animal species suggests a possible zoonotic transmission.
During Nov 2017 and Mar 2019, 48 faecal (n=13) and organ samples (n=35) were collected originating from 25 individuals belonging to 16 different reptile species. All isolated C. difficile strains will be characterized by PCR ribotyping and PCR detection of toxin genes A, B, and the binary toxin genes. Additionally, Multilocus VNTR Analysis (MLVA) and antimicrobial susceptibility testing will be performed. C. difficile was isolated from 15 (29%) out of 51 samples originating from 12/25 (48%) tested individuals. Although molecular characterization has not yet been completed, preliminary results revealed toxigenic ribotypes that have already previously been related to livestock, humans or the environment, e.g. ribotypes 002/2, 005, and 049. Clinical and pathological data suggest that C. difficile can be associated with fatal conditions in reptiles.
This is the first survey on C. difficile in various reptile species proving that reptiles may represent a possible source of infection for CDI in humans.

# H10

## Isolation and Characterisation of Campylobacter-specific bacteriophages

S. Kittler[1], E. Thunhorst[1], S. Steffan[1], G. Shakeri[2], M. Plötz[1], C. Kehrenberg[3]

[1]Institute for Food Quality and Food Safety, University of Veterinary Medicine Hannover,
[2]Department of food hygiene, Ferdowsi University of Mashhad, Iran,
[3]Institute of Veterinary Food Science, Justus-Liebig-University Giessen Member of the "PAC-Campylobacter consortium"

*Keywords: Campylobacter spp., phages, host range*

Campylobacteriosis is one of the most frequent food-borne gastrointestinal diseases worldwide and contaminated poultry meat is the major source of human infections with Campylobacter (C.) spp.. A reduced colonization in primary production of meat is considered to be most effective to combat human campylobacteriosis. In previous in-vivo studies Campylobacter load was significantly reduced by phage application. Subsequent field trials revealed that a broader phage collection is necessary for reproducible reduction under commercial conditions.

In this study, 18 phages were isolated from 288 poultry samples (skin, feces, and caecal content) using C. jejuni NCTC 12662 as a host strain. Samples were tested for plaque formation using the soft-agar overlay technique. Plaques occurred in 17% of all samples and were picked three times to purify phages. Host-ranges of the phages were determined by spot testing using 32 C. jejuni and C. coli isolates.

The phages will be further analysed and complement the current phage collection for the use in broiler chickens.

Supported by the German Federal Ministry of Education and Research

# H11

## Farmed ruminants as sentinels for risk assessment of alimentary infections with the tick-borne encephalitis virus in Baden-Wuerttemberg

Sina Fischer, Jasmin Skuballa

Chemisches und Veterinäruntersuchungsamt Karlsruhe

*Keywords: Tick-borne encephalitis virus; alimentary infections; ruminants; raw milk; sentinels*

Tick-borne encephalitis (TBE) in humans is one of the most important viral tick-borne zoonoses in Europe. Besides the main infection route of TBE virus by tick bite, there is an additional food-borne transmission pathway via raw milk and raw milk products. After infection farmed ruminants only develop a low-level TBE virus viraemia and do not show clinical signs. Though, seroconversion can be observed after infection.
The main objective of the study is the collection of data for risk assessment of human alimentary infections with TBE-virus by investigation of grazing, milk giving ruminants as sentinel animals.
In the first phase of the project natural TBE-foci will be identified by a serological screening of sentinel animals. Therefore, the antibody prevalence of grazing ruminants as sheep, goats and cattle will be investigated by ELISA. In the second phase the status and course of TBE-virus excretion rate in milk in one selected dairy herd will be determined during the seasonal activity of questing ticks by Real-time PCR.
The data obtained are intended to evaluate the risk of human alimentary infections as a result of consumption of virus-containing dairy products.

## H12

### Detection and characterization of *Staphylococcus aureus* in the dairy food chain in Zambia

Bruno S.J. Phiri[1,2,3], Bernard M. Hang'ombe[3], Sara Schaarschmidt[1], Heidi Wichmann-Schauer[1], Alexandra Fetsch[1], Sven Maurischat[1]

[1]German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany
[2]Central Veterinary Research Institute, Lusaka, Zambia
[3]University of Zambia (UNZA), School of Veterinary Medicine, Lusaka, Zambia

*Keywords: S. aureus, MRSA, antimicrobial resistance, food safety, milk*

Raw milk can be a source of pathogenic microorganisms such as *Staphylococcus aureus* (*S. aureus*), including methicillin-resistant *S. aureus* (MRSA). Consumption of raw milk is still very popular in Sub-Saharan African countries. The aim of the SAD-Zambia project is to characterize *S. aureus*/MRSA in the Zambian dairy chain and to reduce health risks to consumers and producers. Around 290 facilities (farms, milk collection centers, traders, processing plants, traditional markets, and supermarkets) were visited in three Zambian provinces to collect app. 1,940 samples. In parallel, more than 400 stakeholders were interviewed to determine milk handling and hygiene practices along the dairy chain in Western, Southern and Lusaka provinces.

In total, 295 isolates were confirmed as *S. aureus* belonging to ≥ 30 known *spa* -types. All isolates derived from the traditional Zambian dairy chain (lacking standardized food safety measures); no *S. aureus* could be isolated from commercially processed (heat-treated) milk and its products. Interestingly, no MRSA was found in any of the samples, but app. 10% of the *S. aureus* isolates were positive for the Panton-Valentine leukocidin. Whole genome sequencing and DNA microarray analysis of selected isolates indicate potential transmission between humans and cows/milk and diverse antibiotic and enterotoxin gene patterns. Hygiene training and improved practices will likely help to reduce *S. aureus* contamination of milk and milk products in Zambia.

# H13

## Knowledge exchange between science and public health: Austausch WIPH – an update

Majella Albrecht[1], Jörg Heusler[2], Uwe Kaiser[3], Birgit Schauer[4], Gabriele Tardel[5], Peter Tinnemann[3], Daniel Zaspel[6]

[1]Landesamt für Arbeitsschutz, Verbraucherschutz und Gesundheit, Abteilung Verbraucherschutz Dezernat V2 - Tiergesundheit, Tierarzneimittel und Tierschutz
[2]Landkreis Vorpommern-Rügen, Fachdienst Gesundheit
[3]Akademie für Öffentliches Gesundheitswesen
[4]Universitätsmedizin Greifswald
[5]Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Lebensmittelhygienischer Dienst
[6]Robert Koch Institut, Abteilung für Infektionsepidemiologie

One Health has been promoted for >20 years to strengthen control and prevention of zoonotic diseases. But barriers remain, particularly limiting intersectoral knowledge exchange between science (WI) and public health (PH). The concept of Austausch-WIPH developed late 2016 initially promoting a crowdsourcing approach to encourage exchange, translation of research and identification of training and research needs. This approach was presented at four conferences, and stakeholder feedback was gathered via an online survey (09/17-09/18; n=110) and 2 participatory workshops (09/18: n=40; 02/19: n=12). The second workshop resulted in two working groups meeting every 6-8 weeks online via the web-application Vitero. In the WG „Content" ten representatives from science and the public health (PHS) and veterinary health sectors (VHS) in MV and BB discuss prioritized pathogens to document collective knowledge and identify information needs and options for enhanced exchange. Results will be presented to a wider audience in webinars (2019: Campylobacter) and workshops (2020: antimicrobial resistance). The WG „Technical implementation" with 7 participants from the VHS, BALVI GmbH and science first focusses on defining technical needs to strengthen exchange within the VHS (federal state-district). In conclusions, targeted exchanges between scientists and PHS and VHS representatives can help identify barriers, needs and potential solutions. One hour every 6-8 weeks is a valuable investment.

# H14

## Evaluation of commercially available serologic tests and in-house ELISA for Leptospira diagnosis

D. Emirhar[1], E. Luge1, K. Nöckler[1], M. Richter[1], S. Rettenbacher-Riefler[2], J. Dreesman[2], R. G. Ulrich[3], N.S. Bier[1], A. Mayer-Scholl[1]

[1]German Federal Institute for Risk Assessment (BfR), Berlin, Germany
[2]Governmental Institute of Public Health of Lower Saxony (NLGA), Hannover, Germany
[3]Friedrich-Loeffler-Institut (FLI), Insitute of Novel and Emerging Infectious Diseases, Greifswald -Insel Riems, Germany

*Keywords: Leptospira, epidemiology, IgG ELISA, MAT*

In the past, leptospirosis outbreaks in Germany occurred among strawberry harvesters, however other vulnerable populations might exist. To identify further risk factors for leptospirosis, prevalences of Leptospira-specific antibodies in the general population and populations at risk (such as forestry workers) need to be compared. Prior to such studies, the validity of the applied diagnostic tests should be evaluated carefully. Therefore, a commercial and an in-house ELISA were evaluated and compared to the gold standard test (microscopic agglutination test (MAT)).
525 sera from i) forest workers from different federal states (n=236), ii) outbreak cases (n=71), iii) suspected clinical cases (n=126), and iv) staff of the German Federal Institute for Risk Assessment collected by medical officers (n=92) were tested by in-house IgG-ELISA, commercial IgG-ELISA Kit and MAT, respectively.
29% of the 525 tested sera were IgG positive using the in-house ELISA, whereas 15% showed a positive result in the commercial ELISA. Only 15% of all samples were positive in MAT. When comparing the two ELISAs, the commercial kit with the MAT and the in-house ELISA with MAT, the Kappa values were 0.31 (fair level of agreement), 0.12 (slight level of agreement) and 0.57 (moderate level of agreement), respectively.
As the MAT is an imperfect gold standard test and defined human reference sera are not available, further statistical analyses will be conducted to determine the sensitivity and specificity of these tests.

# H15

## Crimean-Congo haemorrhagic fever virus in Hyalomma impeltatum ticks from North Kordofan, the Sudan

Lidia Chitimia-Dobler[1], Makarim Habeeb Issa, Malaz Esamalden Ezalden, Idris Ahmed Yagoub, Mohamed Abdalsalam Abdalla, Amel Omer Bakhiet, Sabine Schaper, Ramona Rieß, Patrick Vollmar, Anne Grumbach, Malena Bestehorn-Willmann, Markus Antwerpen, Gerhard Dobler, Yassir Adam Shuaib

[1]Bundeswehr Institute für Mickrobiology

*Keywords: CCHF, Hyalomma impeltatum, Sudan*

Ticks are important parasites from an economic and public health point of views, because of their ability to reduce farm animals' productivity and transmit zoonotic diseases. From January to August 2017, ticks were collected from domestic animals from North Kordofan and Kassala, the Sudan. A total of 2,410 ticks belonging to three genera were morphologically identified. The Hyalomma genus was represented by six species from a total of 998 ticks. The most abundant species was Hyalomma impeltatum ticks (60.5%, 598/998). We found an evidence for Crimean-Congo hemorrhagic fever virus (CCHFV) in Hyalomma impeltatum ticks collected from sheep in North Kordofan in the Sudan. Based on sequencing of the partial S gene, the detected virus belongs to lineage III with the closest similarity to a CCHFV strain from Senegal.

# H16

## Risk assessment of Campylobacter via broiler chicken meat in Germany

Racem Ben Romdhane, Roswitha Merle

Institut für Veterinär-Epidemiologie und Biometrie - Freie Universität Berlin

*Keywords: Risk assessment, Campylobacter, Broiler, Germany*

Campylobacteriosis is a major foodborne zoonosis in developed countries where broiler chicken meat was identified as the main source of human contamination.
The objective of this work was to quantitatively assess the risk of Campylobacter via broiler chicken meat in Germany.
An initial review of published studies on risk assessment of Campylobacter via chicken meat was performed. This allowed selecting relevant modelling structures, and compartments to include in our work.
The project is embedded in a research consortium PAC-Campy, where physicians and veterinarians, molecular and epidemiological researchers focus on prevention and control of campylobacteriosis. Data generated from laboratory and field experiments are integrated in the risk assessment. To achieve our goal, a mechanistic "from farm to fork model" was developed accounting for all steps of chicken meat production. This model allows to evaluate the economic impact and the disease burden of human infection by campylobacter from chicken meat. The model was calibrated using up-to-date German production and consumption data as well as data produced through field and experimental studies conducted within the project research consortium.
This model will allow in a next step to investigate the efficacy and the cost-benefit evaluation of control measure to decrease campylobacter impact on humans.

# H17

## Comparative analysis of the tick-borne encephalitis virus load in ticks

Malena Bestehorn-Willmann[1], Giulia Lemhöfer[2], Ramona Rieß[2], Lidia Chitimia-Dobler[2,1], Ute Mackenstedt[1], Gerhard Dobler[2]

[1]Institute of Zoology, Parasitology, University of Hohenheim, Stuttgart, Germany
[2]Bundeswehr Institute of Microbiology, Munich, Germany

*Keywords: Tick-borne encephalitis virus, Infectious particles, tick*

Tick-borne encephalitis is one of the most important infectious diseases of the central nervous system in Eurasia. The infectious agent, the tick-borne encephalitis virus (TBEV) is a member of the flaviviruses and is transmitted to humans via tick bites or the consumption of raw dairy products. So far, only little quantitative data have been collected as to how many infectious virus particles ticks contain and whether the amount of transmitted virus might influence the severity of human disease.
The TBEV load was determined for every RT-PCR positive sample by plaque assay. The obtained data were analysed by correlating the viral load with the tick specific factors (species, stages), environmental factors (sampling location, altitude and time of sampling) and virus specific factors (CT-values, genetic cluster). Our data show a correlation between the virus load and the sampling location. While the sampling site of Heselbach had an average viral load of 7.58 E+5 plaque forming units, the sampling location of Mühlau had 5.13 E+03. Furthermore, adult ticks have a significantly higher viral load than nymphs and the viral load in female ticks is higher than in males.
Our findings indicate that the tick specific factors seem to play an important role as far as the viral load of the ticks is concerned. Furthermore, the sampling location might be a key factor. The time of sampling seems to be of less importance than expected. The other environmental factors as well as TBEV specific factors warrant further studies and might be of importance in the natural transmission cycle of the TBEV.

# Poster Presentations New and Re-Emerging Zoonotic Diseases

## N01 – N31

# N01

## Ngari virus: a natural Bunyamwera and Batai virus reassortant

N. Cichon, M. Eiden, U. Ziegler, M. H. Groschup

Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

*Keywords: Ngari virus, reassortment, cross-reaction*

Ngari virus (NRIV), Bunyamwera virus (BUNV) and Batai virus (BATV) are arthropod-borne viruses of the Bunyamwera serogroup in the Orthobunyavirus genus of the Peribunyaviridae family. They are characterized by a tri-segmented, enveloped negative-strand RNA genome. Sequence analysis showed that NRIV is a natural reassortant resulting from co-infection of BUNV and BATV, as NRIV possesses the M segment of BATV combined with the S and L segments from BUNV. This reassortment probably led to an increased virulence, which is associated with hemorrhagic fever in humans and ruminants. So far, NRIV was isolated only from sub-Saharan Africa, whereas BATV and BUNV are distributed almost worldwide. The aim of the present study is to develop discriminatory serological and molecular biological diagnostic tools for these three viruses and to determine potential cross-reactions. For this purpose NRIV, BATV and BUNV derived antibody-positive sera will be tested in an ELISA based on the glycoprotein Gc of BATV, BUNV and NRIV. The results from the ELISAs will be verified in specific serum neutralizing tests using BATV, BUNV or NRIV as homologous viruses. Furthermore, a quantitative real-time reverse transcription PCR for simultaneous detection of NRIV, BATV and BUNV will be established and evaluated.

# N02

## Effect of Mesonivirus on arboviral infection in mosquito cells

Eric Agboli[1], Mayke Leggewie[1,2], Stephanie Jansen[1], Anna Heitmann[1], Renke Lühken[1], Daniel Cadar[1], Egbert Tannich[1,2], Jonas Schmidt-Chanasit[1], Esther Schnettler[1,2]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]German Centre for Infection Research (DZIF), Partner site Hamburg-Luebeck-Borstel-Riems, Hamburg, Germany

*Keywords: arbovirus, mesonivirus, infection*

Mesonivirus (MSV) is an insect-specific virus (ISV), which is assumed to infect several mosquito populations worldwide, including mosquitoes that can be vectors to arboviruses of medical importance. This increases the potential for mosquitoes to be co-infected with MSV and an arbovirus. It is, however, unclear if a potential co-infection has any effect on arboviral infection and whether it is similar for different arboviruses. Investigating their interactions in vitro is a vital first step to exploring this possibility.
We chose a selection of arboviruses of different families, namely Usutu virus (USUV; Flavivirus), Bunyamwera virus (BUNV; Orthobunyavirus) and Semliki Forest virus (SFV; Alphavirus), and investigated their interaction with MSV in vitro. Co-infection experiments were conducted on Culex-derived cells and changes in arboviral replication were determined via reporter assay or $TCID_{50}$.
Interestingly, the initial co-infection experiments suggest different effects of MSV on the tested arboviruses. For example, in the case of SFV, MSV infection does not seem to have an effect on viral replication. However, BUNV replication seems to be suppressed, although this trend is not significant.
The data suggest that the interaction between MSV and arboviruses differs depending on the arbovirus family. Follow-up experiments are necessary to verify this observation. This broadens our knowledge of the complex interactions of ISV and arboviruses.

# N03

## Anaplasmosis in horse

Drážovská Monika, Vojtek Boris, Mojžišová Jana, Prokeš Marián

Institute of epizootiology and preventive veterinary medicine, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 040 01 Košice

*Keywords: equine granulocytic anaplasmosis, tick borne diseases, Anaplasma phagocytophilum, horses*

The cause of anaplasmosis, a vector-borne disease, is the G- intracellular bacteria belonging to the Anaplasmataceae family. In Europe, the most commonly occurring species is Anaplasma phagocytophilum, which is also the most important pathogen of this family. The disease occurs in horses, domestic and wild ruminants, dogs and humans. The vector is Ixodes ricinus. In the literature, there are no data about the incidence of A. phagocytophilum in horses in Slovakia. The aim of our work was to determine the seroprevalence of A. phagocytophilum in selected horse breeds in Slovakia. Blood samples were taken from the jugular vein from 104 horses from selected stables in Slovakia from April to October 2018. A. phagocytophilum IFA Equine Antibody Kit (Fuller Laboratories, USA) was used to detect specific antibodies against A. phagocytophilum. The results were analysed using a fluorescence microscope. In the case of a positive response at a 1:80 dilution, the result was verified at a 1:640 dilution. From 104 horses, seropositivity was detected in 41,3%. Based on the increasing prevalence of vector-borne diseases and the zoonotic potential of this pathogen, the finding in the current situation about occurrence of A. phagocytophilum is beneficial, also because Slovakia is one of the countries with appropriate bioclimatic conditions for the main vector.

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# N04

## Histological lesions and virus detection in rats, bank voles and mice infected with variegated squirrel bornavirus 1 (VSBV-1)

Daniel Nobach[1], Kore Schlottau[2], Donata Hoffmann[2], Martin Beer[2] and Christiane Herden[1,3]

[1]Justus-Liebig-Universität, Institut für Veterinär-Pathologie, Gießen, Germany
[2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[3]Center of Mind, Brain and Behavior, Justus-Liebig-University Gießen, Gießen, Germany

*Keywords: animal model, bornavirus, rodents, encephalitis*

Variegated squirrel bornavirus 1 (VSBV-1) was identified as cause of human lethal encephalitis. Since 2015, it has been shown to be present in various exotic squirrel populations in zoos and private husbandries.
For the development of small animal models representing either the reservoir (squirrel) or the accidental host (humans), neonatal and adult rats (n=94), bank voles (n=55) and mice (n=96) were infected with VSBV-1 by different routes (intracerebral, intranasal or subcutan). After three / five months, animals were examined for macroscopic and histological lesions. Virus distribution was analyzed by immunohistochemistry.
Macroscopic lesions were found in none of the animals. Some rats (n=3) and bank voles (n=2) displayed a lymphohistiocytic encephalitis with perivascular cuffs and reactive microglia. Viral antigen was present in neurons and astrocytes in the hippocampus, cerebral cortex, thalamus and brain stem in intracerebrally infected rats (n=3) and bank voles (n=3). Additionally, in one subcutaneously infected bank vole neurons and astrocytes of spinal ganglia and spinal cord harboured viral antigen. No virus antigen was found in peripheral organs.
These results indicate different courses of infection depending on the species, the age and the route of infection. The presence of viral antigen in the CNS reflects a strict neurotropism like in accidental host, but the duration of the infection could influence the results and has to be taken into consideration.

# N05

## Experimental infection of Ixodes ricinus ticks with different Bartonella species via artificial feeding

Nina Król[1], Nina Militzer[2], Ard Nijhof[2], Volkhard Kempf[3], Martin Pfeffer[1], Anna Obiegala[1]

[1] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany
[2] Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[3] Institute for Medical Microbiology and Infection Control, University Hospital, Goethe-University, Frankfurt am Main, Germany

*Keywords: Ixodes ricinus, ticks, arthropod-borne, Bartonellosis, Bartonella henselae*

Background: Ixodes ricinus is the most important vector for arthropod-borne zoonotic pathogens in Europe. However, the vector competence of it for Bartonella spp. is still unclear. This study aims to experimentally compare the vector competence of I. ricinus regarding 3 Bartonella species (B. henselae, B. grahamii, B. schoenbuchensis). Methods: 3 groups of ticks were fed on bovine blood spiked with $10^8$ cfu/µL of B. schoenbuchensis, B. henselae or B. grahamii. Ticks were kept at 80% humidity and 20°C until depletion. DNA was extracted from 8-10 engorged ticks per group and life stage in order to verify Bartonella infection by real-time PCR. Results: Out of 1600 ticks, 547 engorged and depleted. The highest depletion success was reached by ticks feeding on B. henselae-spiked blood (n=234), followed by B. schoenbuchensis (n=174) and B. grahamii (n=139). Ticks feeding on B. henselae were the heaviest (larvae: 0.47mg; nymphs: 2.88mg), followed by B. schoenbuchensis (larvae: 0.43mg; nymphs: 2.83mg) and B. grahamii (larvae: 0.36mg; nymphs: 1.73mg). Altogether, 69.3% of ticks feeding on B. henselae were positive by PCR, followed by 65% for B. schoenbuchensis and 16.6% for B. grahamii. So far, 9.2% of ticks fed on B. henselae have molted, followed by 7.8% for B. grahamii and 5.6% for B. schoenbuchensis. Conclusion: I. ricinus ticks may be infected with Bartonella spp. showing a preference for B. henselae and B. schoenbuchensis in contrast to B. grahamii based upon feeding success.

# N06

## Multi-year emergence of monkeypox virus in wild chimpanzees in Taï National Park

Livia Patrono[1], Kamilla Pléh[1], Caroline Röthemeier[2], Liran Samuni[3], Andreas Sachse[1], Markus Ulrich[1], Andreas Nitsche[4], Roman Wittig[4], Sébastien Calvignac-Spencer[1], Fabian Leendertz[1]

[1]Project Group Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institute
[2]Project Group Epidemiology of Highly Pathogenic Microorganisms, Robert Koch Institut
[3]Department of Primatology, Max Planck Institute for Evolutionary Anthropology
[4]Division of Highly Pathogenic Viruses (ZBS1), Robert Koch Institute

*Keywords: monkeypox; wild great apes; clinical features; viral diversity*

Monkeypox is a zoonotic disease endemic to West and Central Africa. Despite the increasing number of monkeypox virus (MPXV) infections in humans, its ecology in wildlife is far from being understood. Between January 2017 and May 2018, we documented the emergence of MPXV in three communities of wild habituated chimpanzees in Taï National Park, Ivory Coast. Pox-like lesions were evident in some individuals, whereas others only showed respiratory symptoms. Full genome sequencing from the skin of a chimpanzee that succumbed to the infection revealed the involvement of a strain belonging to the West African clade. MPXV DNA was detected in non-invasive samples (feces, fruit wedges and urine) collected from both symptomatic and asymptomatic individuals. By applying hybridization capture prior to Illumina sequencing, we retrieved 12 additional complete viral genomes which revealed a different MPXV in each community. Finally, viable virus was isolated from a fecal sample containing a high viral load. These findings add important evidence to MPXV sylvatic maintenance in the Taï forest and its clinical features in wild great apes.

# N07

## Detection of emergent, re-emergent, and neglected diseases in archived CNS samples of horses from Brazil

Gisele Silva Boos[1], Arnt Ebinger[2], Edson Moleta Colodel[3], Dirk Höper[4], David Driemeier[5], Márcio Botelho Castro[6], José Diomedes Barbosa[7], Markus Eickmann[8], Christiane Herden[1]

[1]Institute of Veterinary-Pathology, Justus-Liebig-University
[2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut
[3]Laboratory of Veterinary Pathology, Universidade Federal do Mato Grosso
[4]Laboratory for NGS and Microarray Diagnostics, Friedrich-Loeffler-Institut
[5]Department of Veterinary Pathology, Universidade Federal do Rio Grande do Sul
[6]Laboratory of Veterinary Pathology, Universidade de Brasília
[7]Veterinary Diagnostics Center, Universidade Federal do Pará
[8]Institute of Virology, Medicine Department, Philipps-Universität-Marburg

*Keywords: neuro-infections, formalin-fixed paraffin-embedded, pathogen identification, molecular biology, metagenomics*

This study aimed to investigate non-suppurative encephalitis and encephalopathies in horses from Brazil, suggestive to have an infectious etiology. The thorough histological examination was complemented by IHC, universal PCRs and metagenomics for detection of unexpected or newly emerging pathogens using formalin-fixed paraffin-embedded (FFPE) material. Thirty-five CNS samples were assessed. An infectious etiology could be determined for 14/35 samples, including rabies virus/coccidia coinfection (1/35), alphavirus (3/35), flavivirus (1/35), EHV-1 (3/35), and coccidia (6/35) detection. Unknown infectious etiology (17/35) and miscellaneous non-infectious reactive and degenerative lesions (4/35) were determined based on history, histological alterations, and negative results with the complementary tests. Metagenomic analysis performed with 10/35 RNA samples confirmed Madariaga virus in one alphavirus case and produced significant hits for Elizabethkingia anopheles in another. Assemblies with Parascaris equorum need further investigation since sample contamination might have hindered a correct assignment of reads in 6/10 cases. Contamination during sample collection, source of material and/or lack of appropriate CNS area might have also hindered the identification of other pathogens. Nevertheless, FFPE material used in this study provided valuable epidemiological information and identification of pathogens significant for animal and human health.

# N08

## Searching for the reservoirs of orthobornaviruses

Vanessa Schulze[1], Kore Schlottau[2], Donata Hoffmann[2], Rainer G. Ulrich[1], Martin Beer[2] and Dennis Rubbenstroth[2]

[1]Friedrich-Loeffler-Institut, Insitute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany
[2]Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald-Insel Riems, Germany

*Keywords: bornavirus, reservoir, encephalitis*

Three mammalian orthobornaviruses are known so far: Borna disease viruses (BoDV) 1 and 2 (species: Mammalian 1 orthobornavirus) and the variegated squirrel bornavirus 1 (VSBV-1; Mammalian 2 orthobornavirus). The bicolored white-toothed shrew (Crocidura leucodon) is known as the natural reservoir of BoDV-1, whereas VSBV-1 was detected in different exotic squirrel species kept in captivity but its natural reservoir remains unknown.
One objective of our investigations within the Zoonotic Bornavirus Consortium (ZooBoCo) is to identify the reservoirs of these viruses. For this purpose, we are following targeted and non-targeted sampling approaches. Small mammals, including rodents and insectivores, are tested by real-time reverse transcription-polymerase chain reactions (RT-qPCRs) targeting either BoDV-1/2, VSBV-1 or a broad spectrum of orthobornaviruses. A non-targeted investigation of approximately 5,000 small mammals from Germany did not show any positive samples. Targeted investigations of shrews in BoDV-1-endemic regions detected BoDV-1 in one of 20 bicolored white-toothed shrews. Sequence comparison confirmed a BoDV-1 sequence of the cluster 2 previously identified in Bavaria in encephalitis cases in horses and humans.
In conclusion, the wildlife reservoir of VSBV-1 still remains unclear, whereas our investigations on BoDV-1 confirmed the bicolored white-toothed shrew as reservoir of BoDV-1.

# N09

## In vivo assessing the effect of Carvacrol on Campylobacter spp. prevalence at broiler chicken

Szott V.[1], Reichelt B.[1], Friese A[1], Roesler U.[1]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany

*Keywords: campylobacter, carvacrol, Seeder-bird model*

Background and objectives: Campylobacter infections still are an issue of major importance worldwide. Different in vivo approaches to reduce Campylobacter infections did not prove sufficient. For this reason, we aim to examine the effect of different non-biosecurity based measures on Campylobacter prevalence in animal trials.
Material and Methods: A Seeder-bird model was established to assess the impact of non-biosecurity based measures on Campylobacter prevalence. Broilers of breed ROSS 308 are fed with standard diet and retained on ground floor with litter and a stocking density of 39 kg/m². In each trial we orally challenge 18 (seeders) out of 90 broilers with $10^4$ colony forming units of a C. jejuni reference strain. Successful colonization of the seeder is checked once by taking cloacal swabs 48 hours after inoculation. During the trial 36 randomly selected untreated broilers (sentinels) are tested for Campylobacter colonization and load by taking cloacal swabs at predetermined intervals. At the end of each trial after necropsy we semiquantitatively analyse the intestinal content (ceacum and colon) for C. jejuni. To examine the effect of carvacrol the treated group was fed daily with 120 mg/kg feed of carvacrol.
Results: Our first results show that the C. jejuni load in the carvacrol treated group (mean value 4,19 log 10 MPN/g) is significantly lower compared to the control group (mean value 5,16 log 10 MPN/g). The final results of the ongoing project will be presented.

# N10

## Survival and transmission dynamics of Campylobacter spp. in broiler farm surroundings

Reichelt B.[1], Szott V.[1], Friese A[1], Roesler U.[1]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany

*Keywords: campylobacter, persistence, environment*

Background and objectives: Persistence in the environment as well as the viable but non-culturable (VBNC) state of Campylobacter spp. still remains an under explored field. We investigate possible directions of transmissions and reservoirs of Campylobacter spp. in the environment of broiler farms. Therefore, Campylobacter-positive broiler farms and their environment are sampled intensively. Campylobacter cultivation is done according to DIN ISO 10272. Isolates are species typed with MALDI-ToF analyses. To select isolates for whole genome sequencing flaA types are determined.
Results: We observed high prevalence of Campylobacter spp. in the investigated barn samples (57%) during summer. In comparison we observed lower amounts during winter (32,5 %). First evidence of cultivable Campylobacter spp. in the environment was detected in water-associated matrices in Summer. During winter Campylobacter could be isolated from different environmental sources. Campylobacter was not cultivable after cleaning and disinfection. Most of the analysed samples were identified as C. jejuni. FlaA- typing revealed predominant flaA types that changed after cleaning and disinfection.
Conclusion: Traces of cultivable Campylobacter in the environment show that there is the necessity for deeper investigations especially the (VBNC) state. Therefore, we plan to include a live/dead discriminatory qPCR method aiming to detect possible VBNC state Campylobacter in this ongoing project.

# N11

## Survival time of Leptospira kirschneri serovar Grippotyphosa in animal urine under different environmental conditions

Nau LH, Obiegala A, Speck S, Pfeffer M

[1]Institute of Animal Hygiene and Veterinary Public Health, University Leipzig, Leipzig, Germany

*Keywords: Leptospira spp., survival time, animal urine*

BACKGROUND:
Leptospirosis is a zoonotic disease with around 1 million cases annually. It manifests in animals and humans with a wide spectrum from subclinical to severe symptoms. Leptospira spp. are transmitted via direct or indirect contact to infectious urine of animals. Thus, the survival time of Leptospira spp. in animal urine outside the host is a major factor influencing the infection risk. Many animals are susceptible to infections with Leptospira kirschneri serovar Grippotyphosa including dogs and cattle which also shed the bacteria via urine.
METHODOLOGY:
In this study the survival time of L. Grippotyphosa was examined in urine of dogs and cattle. Undiluted and diluted urine of these animals was spiked with L. Grippotyphosa. After incubating at different temperatures ranging from 15 to 37°C for various time intervals, reisolation attempts of each sample were done in EMJH-Medium. Subsequent cultures were incubated at 29°C and checked for leptospiral growth for at least 28 days.
RESULTS:
L. Grippotyphosa did not survive in undiluted urine. However, L. Grippotyphosa was still viable after an exposure time of up to 72h in diluted urine.
CONCLUSIONS:
Although the leptospiral transmission depends on shedding through the urine of infected animals, the leptospiral strain used in this experiment was not able to survive in undiluted urine. Thus, maintaining leptospiral infectivity outside the body must depend on fast dilution of the urine in the environment.

# N12

## Relationship searching of hepatitis E virus in domestic swine and human patients

[1]Jackova, A., [1]Dudasova, K., [1]Salamunova, S., [2]Paralicova, Z., [2]Sekula, J., [1]Mandelik, R., [1]Novotny, J., [1]Vilcek, S.

[1]University of Veterinary Medicine and Pharmacy in Kosice, Slovakia
[2]The Faculty of Medicine, University P. J. Safarik in Kosice, Slovakia

*Keywords: hepatitis E virus, human, domestic swine*

Hepatitis E virus (HEV) is the causative agent of hepatitis E. Report of hepatitis E in the EU/EEA countries in 2005–2015 showed tenfold increase in number of cases of hepatitis E virus infection. HEV is a highly variable virus, belongs to the family Hepeviridae, genus Orthohepevirus A, subdivided into seven genotypes. Genotypes HEV-1 and HEV-2 infect only humans. HEV-3, HEV-4 and HEV-7 infect humans and several animal species and are considered to be zoonotic. The objective of this study was to search relations between hepatitis E in human and HEV in domestic swine by phylogenetic analysis. A total 122 pig rectal swabs were obtained from slaughter-age pigs originating from domestic pig farms in eastern Slovakia. Five patients with clinical signs of hepatitis E, hospitalized in the Infectious clinic of Hospital Kosice, provided clinical samples (stool, serum). HEV RNA was detected by RT-PCR in all 5 patients and in 18/122 pigs. Phylogenetic analysis of human and pig HEV sequences based on 242 bp fragment from ORF1 showed that all of them belonged to genotype HEV-3, subtypes 3abchij, except for one human HEV isolate clustered in subtypes 3efg. Nucleotide sequences identity of swine HEV isolates was observed 81.4-100% and high sequences identity (94,2%) of swine and human isolates was observed. The results of this study indicate close relationship of human and swine HEV isolates.
The study was supported by APVV-15-0415 project.

# N13

## Expression and molecular interactions of individual tick-borne encephalitis virus proteins

Veronika J. M. Breitkopf[1,2] and Imke Steffen[1,2]

[1]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover, Germany; [2]Institute for Biochemistry, University of Veterinary Medicine, Hannover, Germany

*Keywords: Tick-borne encephalitis virus, flavivirus, viral protein expression, protein modification, protein-protein interaction*

Tick-borne encephalitis virus (TBEV) belongs to the genus Flavivirus within the family Flaviviridae. The single-stranded positive-sense RNA genome encodes a single open reading frame comprising ten viral genes. Three structural proteins (capsid, precursor membrane and envelope protein) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) are expressed as a single polyprotein that is co- and post-translationally cleaved by viral and host cell proteases. This has limited the study of individual TBEV proteins. To investigate the distribution, function and molecular interactions of each viral protein, all TBEV genes were cloned and expressed individually or with adjacent TBEV sequences. Expression was confirmed by Western blot and cellular localization investigated by immunocytochemistry. Molecular interactions between viral and host cell proteins were studied using the recombinant TBEV proteins. Neighboring sequences affected viral protein processing, glycosylation and trafficking. Oligomerization was observed for prM, E, NS1, NS2B, NS3, NS4A and NS5. Heterotypic interactions of TBEV proteins C and prM as well as NS2B and NS3 were detected by co-immunoprecipitation. Further interactions between viral proteins are being investigated. The recombinant TBEV proteins will be tested for interaction with host factors involved in the replication of related flaviviruses and used to identify new host interaction partners that present potential therapeutic targets.

# N14

## Isolation and characterization of Arcobacter strains derived from human stool samples – results from a prospective German prevalence study (Arcopath)

Vanessa Brückner[1], Markus M. Heimesaat[2], Ulrike Fiebiger[2], Ralf Ignatius[3], Johannes Friesen[3], Marlies Höck[4], Martin Eisenblätter[5], Stefan Bereswill[2], Thomas Alter[6], Greta Gölz[7]

[1]Institute of Food Safety and Food Hygiene, FU Berlin
[2]Institute of Microbiology, Infectious Diseases and Immunology, Charité - Universitätsmedizin Berlin
[3]Medizinisches Versorgungszentrum Labor 28 GmbH
[4]Medizinisches Versorgungszentrum Labor Limbach Berlin GbR
[5]synlab Medizinisches Versorgungszentrum Berlin GmbH
[6]Institute of Food Safety and Food Hygiene, Freie Universität Berlin
[7]Institute of Food Safety and Food Hygiene, Freie Universität Berlin

*Keywords: Arcobacter, human, pathogenesis, antimicrobial susceptibility*

Arcobacter species are discussed as emerging food- and waterborne pathogens causing human gastroenteritis. However, reliable epidemiological data are scare. We therefore performed the first German prospective Arcobacter prevalence study (Arcopath) including 3884 stool samples derived from outpatients collected between 10/2017 and 10/2018. Arcobacter species could be isolated from 0.8 % of specimens. Among the 33 Arcobacter -positive tested samples, A. butzleri was the most prevalent species (n=21), followed by A. cryaerophilus (n=10) and A. lanthieri (n=2). A high genetic diversity within the species was determined by ERIC-PCR. Detection of putative virulence genes was highest in A. butzleri, followed by A. cryaerophilus and A. lanthieri. All A. butzleri and A. lanthieri, but only single A. cryaerophilus isolates induced cytotoxic effects in human HT-29/B6 cells in vitro. Normally distributed MIC values were determined for gentamicin and tetracycline, whereas some isolates displayed elevated MIC values for erythromycin (n=2) and ciprofloxacin (n=5) and several for ampicillin (n=10) and azithromycin (n=13).
Our study reveals i) an Arcobacter prevalence in German outpatients of <1 %, ii) prominent in vitro cytotoxic effects of A. butzleri, but not of A. cryaerophilus, and iii) antimicrobial susceptibilities towards commonly prescribed antibiotic compounds in medicine. However, a more in-depth evaluation of the role of Arcobacter in human disease requires further investigation.

# N15

## Hantavirus and Leptospira spp. infections in European rodents

Kathrin Jeske[1], Stefan Fischer[1], Duygu Emirhar[2], Jose Francisco Ruiz-Fons[3], David Gonzalez-Barrio[4], Petra Strakova[5], Linas Balčiauskas[6], Laima Balčiauskienė[6], Christian Imholt[7], Jens Jacob[7], Rainer G. Ulrich[1]

[1]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Südufer 10, 17493 Greifswald-Insel Riems
[2]Department for Biological Safety, Federal Institute for Risk Assessment, 12277 Berlin, Germany
[3]National Wildlife Research Institute IREC, CSIC-Universidad de Castilla – La Mancha; 13071 Ciudad Real, Spain
[4]SALUVET, Complutense University, Madrid, Spain
[5]Veterinary Research Institute, Hudcova 70, 612 00 Brno, Czech Republic
[6]Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania
[7]Julius Kühn-Institute, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Toppheideweg 88, 48161 Münster, Germany

*Keywords: vole, coinfection, Tula orthohantavirus, Leptospira kirschneri*

Hantaviruses and Leptospira spp. are zoonotic pathogens that are associated with different rodent species. Rodents are persistently infected and shed the pathogens with their excreta. Zoonotic hantaviruses and Leptospira spp. cause disease in human with similar clinical symptoms.
Rodents from Germany, Lithuania and Spain were collected and analyzed by hantavirus-specific reverse transcription-polymerase chain reaction (RT-PCR) targeting the S segment and by conventional lipl32 gene specific PCR for pathogenic Leptospira spp. detection. The determination of hantavirus and Leptospira species was done by sequencing of the obtained RT-PCR products and the analysis of the secY PCR products, respectively.
Tula orthohantavirus (TULV) and Leptospira kirschneri were detected in common voles (Microtus arvalis) from Germany. Hantaviruses were detected in voles from Lithuania and different Leptospira spp. in common voles and Lusitanian pine vole (Microtus lusitanicus) from Spain. Currently, the frequency of TULV and Leptospira spp. coinfection in rodents are being investigated.

# N16

## Novel sandfly-borne phlebovirus from Nepal

Ramesh Pun[1,2,3], Anne Kopp[1,2], Florian Zirkel[2,4], Meghnath Dhimal[5], Ishan Gautam[6], Chop Lal Bhusal[5], David A. Groneberg[3], Christian Drosten[1,2], Ulrich Kuch[3], Sandra Junglen[1,2]

[1] Institute of Virology, Charité -Universitätsmedizin, Berlin, Germany
[2] German Center for Infection Research (DZIF), Berlin, Germany
[3] Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Goethe University, Frankfurt am Main, Germany
[4] Institute of Virology, University of Bonn Medical Centre, Bonn, Germany
[5] Nepal Health Research Council, Kathmandu, Nepal
[6] Natural History Museum, Tribhuvan University, Kathmandu, Nepal

*Keywords: phlebovirus, sandfly, Nepal, SNEV*

Phleboviruses (family Phenuiviridae) are mainly transmitted by mosquitoes, ticks or sandflies. Sandlfy-borne phleboviruses can cause fever, febrile illness and neurological symptoms. The viruses are widely distributed in Eurasia, but no data are available for Nepal. The aim of the study was to test sandflies from Nepal for infections with phleboviruses.
Sandflies (n=152) were collected in the Morang, Dhankuta and Terathum districts in Nepal. Pools (n = 18) of homogenized sandflies were tested for phlebovirus infection by a generic RT-PCR. Viral genome sequencing was performed by generic RT-PCR, primer-walking techniques and next generation sequencing (NGS). Virus isolation was done in mosquito (C6/36) and vertebrate (Vero) cells.
A previously unknown phlebovirus, tentatively named Sandfly Nepal virus (SNEV), was detected in one pool of Sergentomyia spp. sandflies. Almost entire coding sequences of all three segments were obtained (L: 6,198 nt, M: 2,334 nt, S: 482 nt). Phylogenetic analyses of the SNEV RdRp and glycoproteins showed that SNEV was most closely related but distinct to viruses of the Sandfly fever Sicilian group. In contrast, the SNEV nucleocapsid protein grouped with viruses of the Sandfly fever Naples complex. Virus isolation attempts were not successful.
This is the first detection of a phlebovirus in sandflies from Nepal. Further studies are needed to characterize the novel virus, as well as to investigate if it has an impact on human and animal health.

# N17

## Generation of Simian Rotavirus Reassortants with Diverse VP4 and VP7 Genes Using Reverse Genetics

Alexander Falkenhagen[1], Corinna Patzina-Mehling[1], Antje Rückner[2], Nilsa de Deus[3], Hester Gertruida O'Neill[4], Thomas Vahlenkamp[3], Reimar Johne[1]

[1]The German Federal Institute for Risk Assessment, Berlin, Germany
[2]Institute of Virology, Leipzig University, Leipzig, Germany
[3]National Institute of Health, Maputo, Mozambique
[4]University of the Free State, Bloemfontein, South Africa

*Keywords: Rotavirus, reassortment, reverse genetics system, VP4, VP7*

Group A rotaviruses (RVAs) are one of the leading causes for severe gastroenteritis in young animals and humans. The RVA genome consists of 11 dsRNA segments and reassortment events between animal and human strains can contribute to a high genetic diversity. Here, we used a plasmid-based reverse genetics system to investigate the reassortment potential of the genome segment encoding VP4, the outer capsid spike protein. VP4 is a major antigenic determinant, mediates viral entry, and plays an important role in host cell tropism. We were able to rescue reassortants containing VP4 from porcine, bovine, bat, pheasant, or chicken RVA strains in the backbone of simian strain SA11. In addition to VP4, the outer capsid glycoprotein VP7 also represents a major antigenic determinant. Using the same reverse genetics system, we were able to generate reassortants with VP4 or VP7 derived from primary human RVA strains circulating in Africa. All reassortants could be stably passaged and induced cytopathic effects in cell cultures, but analysis of growth kinetics revealed marked differences in replication efficiency. Our results show that the genome segments encoding VP4 or VP7 have a high reassortment potential, resulting in replication-competent reassortants with new genomic, growth, and potentially antigenic features. Our results also indicate that the reverse genetics system may be useful for the generation of vaccine candidate strains in future.

# N18

## Establishment of a plasmid-based reverse genetics system for investigation of the zoonotic potential of rotaviruses of poultry

Corinna Patzina-Mehling, Alexander Falkenhagen, Eva Trojnar, Reimar Johne

German Federal Institute of Risk Assessment

Group A rotaviruses (RVA) are important gastrointestinal pathogens, which are widely distributed in humans, mammalian animals and birds. Reassortment between human and mammalian animal strains is common, but corresponding data on avian strains are rare. We recently created a simian RVA (strain SA11) carrying a chicken RVA-VP4 using a helper virus-dependent reverse genetics system (RGS), indicating that avian/mammalian RVA reassortants can be viable.
To further investigate the zoonotic potential of avian RVAs, a more flexible helper virus-free RGS should be established here for the chicken RVA strain Ch2G3 based on the available system for the SA11 strain. All 11 Ch2G3 genome segments were cloned into separate plasmids containing regulatory sequences for RNA transcription. To screen for the reassortment capacity, transfection experiments with different combinations of SA11/Ch2G3 plasmids were performed. By this, we successfully reproduced the SA11/Ch2G3-VP4 reassortant and additional reassortants carrying other single Ch2G3 genome segments were generated. The replication kinetics of these viruses will be further characterized and screened for potential adaptive mutations after serial passaging in cell culture.
The results should give insights into the requirements for avian/mammalian RVA reassortment. In addition, the risk of zoonotic transmission of RVA from poultry to mammals and the possibility of the emergence of novel reassortants should be assessed.

# N19

## The rat from an aircraft: Investigations for multiple pathogen detection

Elisa Heuser[a], Arnt Ebinger[b], Silva Holtfreter[c], Andreas Zautner[d], René Ryll[a], Stephan Drewes[a], Beate Matzkeit[a], Bernd Hoffmann[b], Dirk Höper[b], Markus Keller[a], Allison Groseth[f], Gottfried Wilharm[e], Daniel Mrochen[c], Anna Obiegala[g], Frank Doss[h], Tobias Eisenberg[i], Sandra Niendorf[j], Sindy Böttcher[k], Michael H. Kohn[l], Charlotte Schröder[m], Joachim Reetz[n], Kati Franzke[o], Eric Ehrke-Schulz[p], Martin Pfeffer[g], Katja Schmidt[q], Martin Beer[b], Rainer G. Ulrich[a,r,*]

[a] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[b] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Diagnostic Virology, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[c] Department of Immunology, University Medicine Greifswald, 17475 Greifswald, Germany
[d] Institute of Medical Microbiology, University Medicine Göttingen, Kreuzbergring 57, 37075 Göttingen, Germany
[e] Robert Koch Institute, Wernigerode Branch, Burgstr. 37, 38855 Wernigerode, Germany Friedrich-Loeffler-
[f] Institut, Federal Research Institute for Animal Health, Group – Arenavirus Biology, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[g] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, An den Tierkliniken 1, 04103 Leipzig, Germany
[h] Clean Frank Doss GmbH, Kampstraße 27, 16792 Zehdenick, Germany
[i] Hessian State Laborytory, Schubertstraße 60 - Haus 13, 35392 Gießen, Germany
[j] Robert Koch-Institute, Department of Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Seestraße 10, 13353 Berlin, Germany
[k] Robert Koch-Institute, Regional Reference Laboratory of the WHO/Europe for Poliomyelitis, Seestraße 10, 13353 Berlin, Germany
[l] Rice University, BioSciences, Houston, TX
BioSciences MS-170, Rice University, 130 Anderson Biology, P.O. Box 1892, Houston, Texas, 77251-1892, USA
[m] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[n] German Federal Institute for Risk Assessment, Diedersdorfer Weg 1, 12277 Berlin, Germany
[o] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Infectology, Südufer 10, 17493 Greifswald-Insel Riems, Germany
[p] Department of Virology and Microbiology, Center for Biomedical Education and Research, University of Witten/Herdecke, Stockumer Strasse 10, 58453 Witten, Germany
[q] Microbiological Diagnostics, German Cancer Research Center Heidelberg, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[r] German Center for Infection Research (DZIF), Partner Site Hamburg-Luebeck-Borstel-Insel Riems, Germany

*Keywords: rat, aircraft, pathogens, workflow, investigations*

Rats are reservoirs for a variety of zoonotic pathogens like cowpox virus, Seoul orthohantavirus and Leptospira spp., which can cause serious diseases in humans. Additionally, non-zoonotic pathogens such as rat hepacivirus and polyomaviruses were found in rats.
In April 2017, a passenger noticed the presence of a rat in an aircraft during the flight. After landing in Berlin, the rat was localized, euthanized and subsequently dissected. Various tissue samples were taken for different pathogen investigations. The animal was identified as a Black rat (Rattus rattus) by standard cytochrome b-PCR and subsequent sequencing.
This event was taken as occasion to establish a standard workflow for rodent-borne pathogen detection. Open-view methods were conducted, including cultivation and characterization of bacteria, high throughput sequencing, electron microscopy, microarray, and multiplex serology investigations. The pathogen-specific methods targeted 20 viral and eleven bacterial pathogens.
Cultivation experiments showed the presence of five bacterial and two fungal species. A methicillin-sensitive Staphylococcus aureus strain (MSSA-CC45) was detected by a specific multiplex PCR and high throughput sequencing investigations revealed two novel picobirnaviruses.
In conclusion, a few zoonotic and non-zoonotic pathogens were detected in the rat but no evidence for other pathogens is presumably, as they would have been found with the workflow investigations, especially open view methods.

# N20

## Generation of 3D cell culture models (spheroids/organoids) to study interactions of zoonotic agents with the bovine intestinal mucosa

Jan-Dirk Haeger[1], Christian Berens[1], Soroush Sharbati[2], Christian Menge[1]

[1]Friedrich-Loeffler-Institut/ Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis
[2]Department of Veterinary Medicine, Institute of Veterinary Biochemistry, Freie Universität Berlin

*Keywords: Enteral organoids, hanging drops, matrigel*

3D cell cultures resemble the histological structure of tissues more closely than monolayers. The aim of our study is to establish 3D cultures which consist of various intestinal cell types isolated from cattle. Hanging drops (2 days) or matrigel (10 days) approaches were utilized to generate the 3D cultures. Primary cells (fibroblasts) and stable cell lines (FKD-R) were used as starting material, as well as primary colonic crypts. Different combinations of cells were employed in the hanging drops assays. We also approached the isolation of primary bovine intestinal crypt epithelial cells (pCEC) by use of enzymatic digestion.
When crypts were seeded in matrigel, they formed organoids (1000 µm) which were demarked to the outside, as assessed by light microscopy. Hematoxylin-Eosin staining indicated that the organoids were made up of intestinal epithelium. In hanging drops, all cell combinations yielded cell spheroids. The spheroids/organoids survived harvest as they were mechanically stable, thereby providing proof for the formation of in vitro microtissues. The protocols for the continuous pCEC (2D) culture require optimization.
The established bovine intestinal colonic 3D-culture models will be employed to analyse the interplay of foodborne zoonotic agents transmitted from cattle to humans (e.g. Shiga toxin-producing Escherichia coli [STEC]).

# N21

## Diverse novel phleboviruses in sandflies from the Panama Canal area, Central Panama

David P. Tchouassi[1], Marco Marklewitz[2], Edith Chepkorir[3], Florian Zirkel[1], Sheila B. Agha[4], Caroline C. Tigoi[1], Edith Koskei[1], Christian Drosten[5], Christian Borgemeister[2], Baldwyn Torto[6], Sandra Junglen[7], Rosemary Sang[1],

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya
[2]Charité-Universitätsmedizin Berlin, Berlin, Germ
[3]German Center for Infection Research, Berlin, Germany
[4]Biotest AG, Dreieich, Germany
[5]Center for Virus Research, Kenya Medical Research Institute, Nairobi
[6]German Center for Infection Research, Berlin, German
[7]Center for Development Research, University of Bonn, Bonn, German
[8]Charité-Universitätsmedizin Berlin, Berlin, Ger
[9]International Centre of Insect Physiology and Ecology, Nairobi, K

*Keywords: Phlebovirus, sandfly, Panama Canal, virus taxonomy*

Sandfly-transmitted phleboviruses are responsible for febrile illness and infections of the nervous system in humans of Europe, Africa, and the Americas. The genus Phlebovirus (family Phenuiviridae) comprises 57 viruses that are grouped into 9 species-complexes based on serological reactivity. Our study aims to assess the genetic diversity of sandfly-transmitted phleboviruses in forest habitats in Central Panama. In total, 13,807 sandflies were collected comprising 8 species. We detected several strains of 5 previously unknown viruses showing maximum p-distance of 55-22% to the RdRp of phleboviruses. Viruses were named La Gloria (LAGV), Mona Grita (MOGV), Peña Blanca (PEBV), Tico (TICV), and Tres Almendras virus (TRAV). Inferred phylogenies and p-distance-based analyses revealed that PEBV groups with Bujaru phlebovirus, TRAV with Candiru phlebovirus, and MOGV belongs to the suggested Icoarci phlebovirus species. LAGV and TICV seem to be distant members of Bujaru phlebovirus. Using these novel sequences and all currently available phleboviral sequences, we sought to establish genetic-based species demarcation criteria for the genus Phlebovirus. Our analyses suggest differentiating these viruses by at least 5% amino acid distance in their RdRp proteins. Our study shows that sandflies from remote and biodiverse habitats harbour multiple unknown phleboviruses. Future studies should assess their geographic distribution and their potential to cause disease in animals and humans.

# N22

## Human Puumala orthohantavirus cases in a company of northwestern Germany

S. Drewes[1], C. Princk[2], M. Mylius[2], K. Schlinkmann[2], M. Saathoff[3], J. Freise[3], B. Tenner[4], S. Weiss[4], J. Esser[5], M. Runge[3], R.G. Ulrich[1], J. Dreesman[2]

[1]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany;
[2]Governmental Institute of Public Health of Lower Saxony, Department: Microbiology, Infection Protection, Hospital Hygiene and Epidemiology of Infectious Diseases, Hannover, Germany;
[3]Lower Saxony State Office for Consumer Protection and Food Safety, Wardenburg, Germany;
[4]Institute of Virology, Charité-Universitätsmedizin, Berlin, Germany;
[5]Laboratory medical practice Osnabrück, Osnabrück, Germany

*Keywords: Puumala orthohantavirus, human cases, Myodes glareolus, hantavirus outbreak*

In 2001, laboratory diagnosed acute hantavirus infection and viral haemorrhagic fever became notifiable in Germany. Besides the occurrence of high endemic regions in western and southwestern Germany, recorded hantavirus disease cases are subject to large annual fluctuations culminating in so-called hantavirus outbreak years. The main causative agent is Puumala orthohantavirus (PUUV) harbored by its natural host, the bank vole (Myodes glareolus).
In the end of the outbreak year 2017, employees of a distribution company in the federal district Grafschaft Bentheim showed typical signs of hantavirus disease. In early 2018, bank vole trappings were conducted at the company. Trapped rodents and hospitalized employees were tested by IgG-ELISA and by one-step PUUV S and L segment RT-PCRs. In addition, a questionnaire survey among the employees was performed.
According to the questionnaire, a rodent infestation of the company building was observed in late 2017. In total, 5 of 8 employees identified the invasive rodents as bank voles. Out of 48 trapped bank voles 11 (23%) were tested positive for PUUV by IgG-ELISA and 13 (27%) were positive by S segment RT-PCR. From one hospitalized employee a partial PUUV L segment sequence could be generated. The human PUUV L segment sequence was identical to bank vole L segment sequences obtained from the company compound.
In conclusion, this One Health investigation gave molecular epidemiological evidence for PUUV infection of employees of a company.

# N23

## Survival time of Leptospira kirschneri serovar Grippotyphosa on strawberries

Duygu Emirhar, Enno Luge, Nadja Bier, Karsten Nöckler, Martin Richter, Anne Mayer-Scholl

German Federal Institute for Risk Assessment

*Keywords: Leptospirosis, strawberries, Leptospira, survival*

In two recent leptospirosis outbreaks among strawberry harvesters in Germany, common voles (Microtus arvalis) infected with Leptospira kischneri serovar Grippotyphosa were identified as the most likely outbreak source. In an univariate analysis, eating unwashed strawberries was one of the risk factors associated with Leptospira infection. This led to the question, if Leptospira spp. can survive on strawberries long enough to pose a public health concern. Therefore, the aim of this study was to evaluate the survival time of L. kirschneri serovar Grippotyphosa on strawberries under varying temperatures and incubation time combinations.
Survival time of L. kirschneri serovar Grippotyphosa strain Moskvain was tested by combining each incubation time (2h, 4h, 6h and 8h) with each temperature condition (15°C, 20°C and 25°C) in triplicate. A wash protocol was developed and recovered Leptospira in the washing suspension were determined by qPCR, dark field microscopy and culturing.
After washing, nearly 100% of the spiked Leptospira were recovered and detected by qPCR and 92% by counting using a Thoma cell chamber. Viable L. kirschneri were identified in all samples at 25°C and an incubation time of 2h, but only in 11% of the samples kept at 15°C. After 6h, viable Leptospira were found in 3/9 cultures at 15°C, 1/9 cultures at 21°C and 2/9 cultures at 25°C. After 8h incubation time, viable Leptospira could not be identified in culture or by dark field microscopy.

# N24

## Luminex-based multiplex assay for the detection of antibodies against different henipaviruses and other relevant agents in pigs

M. Penning[1], K. Fischer[1], A. Wade[3], C. Mbanwi Mbu'u[2], W. Fon Mbacham[4], S. Diederich[1], M.H. Groschup[1], A. Balkema-Buschmann[1]

[1]Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[2]Department of Microbiology, Faculty of Science, the University of Yaoundé I, Yaoundé, Cameroon
[3]National Veterinary Laboratory, Garoua and Yaoundé, Cameroon
[4]Laboratory for Public Health Research and Biotechnologies, the Biotechnology Centre – the University of Yaoundé I, Yaoundé, Cameroon

*Keywords: Ghana Virus, Eidolon helvum, Henipavirus, G-proteins and Luminex*

Ghana virus (GhV) is a paramyxovirus within the genus Henipavirus with unknown zoonotic potential, which is closely related to the BSL4 agents Hendra (HeV) and Nipah virus(NiV), that have been reported to cause deadly infection in horses, pigs and humans in Australia and South-East Asia. Ghana virus was first identified in a spleen sample of the species Eidolon helvum in Kumasi, Ghana. So far, all attempts to isolate the virus from tissue samples or to rescue a recombinant GhV were unsuccessful.
Based on the serological cross-reactivity between henipaviruses circulating in Africa and the Australasian region by using serological assays based on HeV and NiV glycoprotein G, henipavirus specific antibodies have been detected in fruit bats, pigs, horses and humans in a number of Sub-Saharan African countries recently.
We therefore developed a Luminex technology based multiplex immunoassay to detect antibodies against the above mentioned henipavirus G proteins, HeV, NiV and GhV G. This assay is based on antigen-coated magnetic beads in a fluid system to test against several agents in a multiplex format. Validation of the assay is currently being performed on pig samples from Sierra Leone. This assay will then be expanded by adding the antigens of other relevant viruses, i.e. Ebola virus. This improves the diagnostic capability for henipaviruses and other disease agents in field samples from Cameroon.

# N25

## Influence of genetic adaptation to vector populations on arbovirus emergence and spread

Frederic Gusmag[1,2], Martin Rudolf[3], Anna Heitmann[3], Stephanie Jansen[3], Marlis Badusche[3], Mayke Leggewie[3], Robert Meineke[2], Benjamin Brennan[4] Claudia Schulz[2], Stefanie Becker[1,2]

[1] Institute of Parasitology, University of Veterinary Medicine Hannover (TiHo), Germany
[2] Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover (TiHo), Germany
[3] Bernhard-Nocht-Insitute for Tropical Medicine (BNITM), Hamburg, Germany
[4]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow

*Keywords: Orthobunyaviruses, Arboviruses, Reassortment, Reverse Genetics, Viral Adaptation*

Arthropod-borne viruses usually alternate between an insect vector and a vertebrate host, which postulates rather quick adaptation to new environments. Bunyamwera orthobunyavirus (BUNV) and Batai orthobunyavirus (BATV) are rather successful in their ability to adapt to new hosts; hence, they are able to infect a wide range of different vertebrates.
Previous in-vitro results (co-infection and growth kinetic studies) show viral reassortment of BUNV and BATV only in mammalian cells (BHK-21) with faster viral replication than their respective parental viruses. However, in insect cells (U4.4, C6/36) no viral reassortment was observed and only instable 4-segmented viruses were obtained. Furthermore, in insect cells selected stable L and M segment reassortants showed an impaired replication compared to the parental viruses. First infection experiments in Aedes aegypti show infection rates between 1,7% (for the selected reassortants), and 33% (BUNV) with no significant differences between the 4 tested viruses. Overall, the infection rates had their peak 7 dpi and decreased up to 21 dpi.
Focus of this study is the establishment of reverse genetic systems for BATV in addition to the established BUNV reverse genetic system to assess the adaptation of these viruses to their respective vectors and hosts, regarding vector specificity and potential vector restrictions. Another aspect will be viral reassortment of Orthobunyaviruses and their adaptation to mammalian hosts and insect vectors.

# N26

## Target tissues of the Puumala orthohantavirus (PUUV) in its natural host, the bank vole (Myodes glareolus)

Elfi Schlohsarczyk[1], Reiner Ulrich[2], Jan Schinköthe[2], Julia Sehl[2], Jens Peter Teifke[2], Susanne Röhrs[3], Stefan Fischer[4], Rainer Ulrich[4], Christiane Herden[5]

1 Institute of Veterinary-Pathology, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Giessen, Germ
[2]Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[3]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[4]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[5]Institute of Veterinary-Pathology, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Giessen, Ger

*Keywords: Puumala orthohantavirus, tissue tropism*

Background and objectives: PUUV is one of the most important hantaviruses in Europe, with the bank vole as reservoir. PUUV transmission between rodents and to humans happens mainly via inhalation of virus-containing aerosols. Affected people can show flu-like symptoms up to renal failure, whereas infected bank voles do not exhibit any clinical signs. The aim of this study is to describe the tissue tropism of PUUV in its natural reservoir.
Materials and methods: Naturally infected laboratory and wild bank voles were necropsied looking for gross pathologic findings, and subsequently tested for hantavirus-RNA by RT-PCR using lung tissue. Haematoxylin-eosin stained slides of the PUUV-RNA positive animals were screened for histological alterations and immunohistochemistry (IHC) was performed using a polyclonal pig-anti-hantavirus-nucleocapsid-protein-antibody. IHC positive tissues and additional secretory and excretory organs were tested by PUUV RT-qPCR and in situ-hybridization (ISH) detecting hantaviral mRNA.
Results: IHC demonstrated viral antigen in brain, heart, lung, tongue, stomach, parotid gland, pancreas, liver and kidney. Presence of mRNA was revealed in these organs as well as in the intestine, adrenal gland, brown fatty tissue and in the submaxillary salivary gland. Notably, IHC and ISH-positive tissues had no gross or histopathological lesions.
Conclusions: The results indicate secretory and excretory organs as main target tissues for viral shedding and transmission.

# N27

## Carcass monitoring as a disease surveillance tool in the Dzanga Sangha Protected Areas (DSPA) of the Central African Republic

Tobias Gräßle[1,3], Therese Löhrich[1,2], Frédéric S. Singa Niatou[2], Sébastien Calvignac-Spencer[1], Ilka Herbinger[3], Terence Fuh Neba[2], Fabian H. Leendertz[1]

[1]Epidemiology of highly pathogenic microorganisms, Robert Koch-Institute, Berlin, Germany
[2]World Wide Fund for Nature (WWF) Central African Republic (CAR), Bangui, CAR
[3]World Wide Fund for Nature Germany, Berlin, Germany

*Keywords: Disease surveillance, carcass monitoring*

Background and Objectives: Rapid diagnostics are critical for early disease outbreak recognition and effective containment of epidemics. Looking at zoonoses the ideal scenario is to detect the emergence of a disease in the animal population before spillover into human populations. This is particularly important in high risk areas with known occurrence of highly pathogenic zoonoses and with frequent exposure of the local population to wildlife. One such area is the Congo basin, where fatalities due to Ebola, monkeypox and anthrax have been reported and where bush meat hunting and consumption is common. Therefore, the WWF Germany and the WWF CAR, in close collaboration with the Robert Koch-Institute, implemented systematic carcass monitoring and disease surveillance in the DSPA.
Materials and Methods: Every animal found dead in the forest is sampled under high safety standards wearing according personal protective equipment. These samples are tested immediately in an on-site laboratory via PCR. Every carcass at least gets screened for ebolaviruses and other filoviruses, Bacillus anthracis and B. cereus biovar anthracis as well as monkeypox virus. Validation of positive PCR results and further pathogen characterization is conducted on-site based on MinION sequencing. The project is performed in close collaboration with national reference laboratories and all cases are communicated to the according national health authorities.

# N28

## Hazara orthonairovirus as a model for CCHFV infections in ruminants

Julia Hartlaub, Felicitas von Arnim, Markus Keller, Maryna Somova, Martin Eiden, Ute Ziegler, M. H. Groschup

Institute of Novel and Emerging Infectious Diseases (INNT), Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany

*Keywords: Hazara orthonairovirus, pathogenesis, CCHFV*

*Hazara orthonairovirus* (HAZV) is a tick-borne arbovirus which belongs to the genus *Orthonairovirus* within the order *Bunyavirales*. The natural hosts are unknown and no disease has been reported in vertebrates (incl. humans) so far. However, this virus is of interest because it is a close relative to *Crimean-Congo hemorrhagic fever orthonairovirus* (CCHFV) which is known to cause fatal diseases in humans. Belonging to the same serogroup as CCHFV we are studying HAZV as potential model virus for this high-consequence virus. Whereas CCHFV is a BSL4-agent, HAZV studies can be done under BSL2-conditions.
Little is known at present about HAZV infections and the corresponding pathogenesis in ruminants. Therefore, we have infected domestic sheep with HAZV to prove their susceptibility. By analyzing swab samples virus replication and shedding by oral and excremental routes were analyzed at different time points post infection. Isolated RNA from blood samples as well as from organs after necropsy show the course of viral replication and distribution within the animal. Furthermore, blood and organ material from these animals are used as reference materials, e.g. for the development of diagnostic assays. Major emphasis will be put on the establishment of ELISA systems and neutralization assays to evaluate the extent of cross-reacting antibodies.

# N29

## *Culex torrentium*: A Potent Vector for the Transmission of West Nile Virus in Central Europe

Stephanie Jansen[1], Anna Heitmann[1], Renke Lühken[1], Mayke Leggewie[1], Michelle Helms[1], Marlis Badusche[1], Giada Rossini[2], Jonas Schmidt-Chanasit[3], Egbert Tannich[1,4]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Unit of Microbiology, Regional Reference Centre for Microbiological Emergencies (CRREM)
[3]Bernhard Nocht Institute for Tropical Medicine, Hamburg Germany; University of Hamburg
[4]German Centre for Infection Research (DZIF), partner site Hamburg-Luebeck-Borstel-Riems, Hamburg, Germany

*Keywords: West Nile Virus; Culex torrentium; Arbovirus; Vector competence*

The continuous circulation of West Nile virus (WNV) in Central, South and East Europe and its recent detection in several dead birds and two horses in Germany highlights the need for information on WNV vector competence of mosquitoes from Central Europe. Therefore, three common Culex species (Culex pipiens biotype pipiens, Culex pipiens biotype molestus and Culex torrentium) from Germany were orally infected with WNV and kept at 18 °C, 21 °C, 24 °C or 27 °C for 14 or 21 days post infection (dpi). Thereafter viable WNV was present in the saliva in all tested taxa, but only at incubation temperatures of 24 °C or 27 °C and predominantly at the extended incubation period of 21 dpi. Highest transmission efficiency rates of 17 % (24 °C) and 24% (27 °C) were found for Cx. torrentium. Culex p. pipiens and Cx. p. molestus showed low transmission efficiencies with a maximum of only 3%. Consequently, temperatures above 21 °C support transmission of WNV, which matches the predominant distribution of human WNV cases around the Mediterranean Sea and in South-East Europe. Culex torrentium has been identified as a potent vector for WNV in Central and Northern Europe, which highlights the need for surveillance of mosquito-borne viruses north of the Alps.
[Jansen, S. et al. Culex torrentium: A Potent Vector for the Transmission of West Nile Virus in Central Europe. Viruses **11**, (2019).]

# N30

## Internal gene cassette of Eurasian H9N2-type avian influenza A virus (IAV) strains alters viral replication and pathogenicity of highly pathogenic H5N8-type avian IAV in mammals

Ahmed Mostafa[1, 2], Elsayed M. Abdelwhab[3], Thomas C. Mettenleiter[3], Stephan Pleschka[1]

[1]Institute of Medical Virology, Justus Liebig University Giessen, Schubertstrasse 81, 35392 Giessen, Germany
[2]Center of Scientific Excellence for Influenza Viruses, National Research Centre, Egypt
[3]Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany

*Keywords: Avian influenza viruses, reassortment, pathogenicity*

**Background and objectives:** Humans are susceptible to infection with influenza A, B and C viruses. Influenza A viruses (IAV) represent circulating pathogens that cause annual epidemics and occasional pandemics. In parallel, several high pathogenic avian influenza viruses (HPAIV) and low pathogenic avian influenza viruses (LPAIV) have (occasionally) crossed the species barrier from birds to mammals/humans upon genetic reassortment (frequently with LPAIV/H9N2 strains) and/or adaptive mutations. Increasing evidences show establishment of stable lineages of HPAIV/H5N8 and LPAIV/H9N2 viruses in chickens worldwide - especially Egypt (EGY) and Germany (GER). This raises concerns that reassortment between these highlighted strains could generate novel viruses with the ability to cross the species barrier.
**Materials and methods:** Herein, we developed reverse genetics systems to generate the three studied wildtype strains and their different reassortants for in vitro and in vivo characterizations
**Results and conclusion:** For early risk estimation we investigated the impact of genetic exchange between intensively circulating LPAIV/H9N2$_{(EGY/GER)}$ and HPAIV/H5N8$_{(GER)}$. Based on our results, we discuss the influence of specific reassortments on the zoonotic potential of HPAIV/H5N8 in mammals in vitro and in vivo.

# N31

## Stability of hepatitis E virus against different pH values

Alexander Wolff[1], Thiemo Albert[2], Reimar Johne[1]

[1]German Federal Institute for Risk Assessment, Berlin, Germany
[2]Institute of Food Hygiene, University of Leipzig, Germany

*Keywords: hepatitis E virus, pH-stability, inactivation*

Hepatitis E virus (HEV) is a causative agent of a human hepatitis, with increasing numbers of notified cases in Europe. This zoonotic virus can be transmitted from pigs and wild boars through direct contact, food and the environment to humans. However, the distinct risks of infection by the specific transmission routes or distinct food types are not known so far, mainly because of the lack of data on the persistence of HEV infectivity under different physico/chemical conditions.

Here, the inactivating effects of different pH values should be investigated, with a special focus on those conditions usually applied in food production. For measurement of HEV infectivity, the cell culture-adapted HEV genotype 3c strain 47832c was used. The cell culture system was further optimized by application of different growing conditions and virus concentration methods. A first series of experiments investigated the HEV stability after treatment at pH 1 to 10 at room temperature for 3 hours. As a result, only little decrease in infectivity was demonstrated after treatment with pH 2 to 9, but nearly complete virus inactivation at pH 1 and 10. Currently, treatment with lactic acid at conditions usually applied in raw sausage production is tested.

The results indicate a high stability of HEV against a broad range of different pH values. The study should help to identify food showing a high risk for virus transmission and define effective inactivation methods in future.

# Poster Presentations Diagnostics

## D01 – D10

# D01

## Commercially available Rapid Immunochromatographic Test kits (LFDs) for rabies diagnosis in brain material fail in interlaboratory comparison

Antonia Klein, Thomas Müller, Conrad Freuling

Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Institute of Molecular Virology and Cell Biology, WHO Collaborating Centre for Rabies Surveillance and Research

*Keywords: rabies, diagnostic tools, lateral flow devices*

Rabies as one of the neglected zoonotic diseases causes approximately 59.000 human deaths per year, primarily affecting developing countries in Asia and Africa. Insufficient surveillance is hampering adequate medical intervention and is driving the vicious cycle of neglect. Laboratory confirmation of the disease is limited as there are e.g. logistical hurdles to transport samples to centralized laboratories. Therefore, there is a need for user-friendly and low-cost reliable diagnostic tools. Lateral flow devices (LFD) offer an alternative to conventional diagnostic methods and may strengthen control efforts in low-resource settings. Within a multi-centered study, we compared five different commercially available LFDs regarding their diagnostic sensitivity and their agreement with standard rabies diagnostic techniques. Our evaluation was based on an interlaboratory comparison between different OIE and FAO reference labs using a broad panel of samples. The sensitivities ranged from 0% up to 61% depending on the type of LFD, however with a variation between the different laboratories. These unsatisfying findings corroborate a previous study and indicate a persistent lack of appropriate test validation and quality control. At present, the tested kits are not suitable for in field use for rabies, especially not for suspect animals with human contact and their distribution is careless, as it may result in human casualties.

# D02

## Differences in susceptibility testing for sulfamethoxazole-trimethoprim among clinical Staphylococcus aureus isolates

A. D. Scholtzek[1], S.-K. Klein[2], S. D. Stöckle[2], I. Eichhorn[1], B. Walther[3], A. T. Feßler[1], D. Hanke[1], H. Gehlen[2], A. Lübke-Becker[1], S. Schwarz[1]

[1]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Germany
[2]Equine Clinic: Surgery and Radiology, Freie Universität Berlin, Germany;
[3]Robert-Koch Institute, Berlin, Germany

*Keywords: sulfamethoxazole-trimethoprim, antimicrobial susceptibility testing, Staphylococcus aureus*

**Background and objectives:** Antimicrobial susceptibility testing (AST) with an automated testing system (AS1) and broth microdilution (BMD1) showed differing results regarding sulfamethoxazole-trimethoprim (SXT) for Staphylococcus aureus. These findings were further investigated.
**Material and methods:** S. aureus isolates (n=19) from horses revealed differing SXT susceptibility results. Therefore, a second AS (AS2) and further CLSI approved methods were tested: two BMD tests, including a commercial plate (BMD2), an inhouse set-up (BMD3) for sulfisoxazole (SUL) and trimethoprim (TMP) separately, and disk diffusion (DD) for SXT, SUL and TMP. Respective resistance genes were deduced from whole genome sequences.
**Results:** AS1 identified all isolates as SXT-resistant, whereas the BMD1 classified only three isolates as resistant and 16 as susceptible. AS2 classified 17 as resistant and two as susceptible and BMD2 classified all but one as resistant. DD identified three as SXT-resistant, eleven as SXT-intermediate and five as SXT-susceptible. For SUL, BMD3 classified 16 as susceptible and three as resistant, whereas in DD all were susceptible. In BMD3 and DD, all isolates were TMP-resistant. All isolates harbored a TMP resistance gene (dfrG [n=3] or dfrS1 [n=16]).
**Conclusion:** SXT is used in human and veterinary medicine. Therefore, a correct classification of the AST results of bacterial pathogens is important, keeping in mind that different methods can reveal different results.

# D03

## Development of a broth microdilution method for biocide susceptibility testing of bacterial isolates using four reference strains

A. R. Schug[1], A. T. Feßler[1], A. Bartel[2], A. D. Scholtzek[1], M. Meurer[3], J. Brombach[1], V. Hensel[4], S. Schwarz[1]

[1]Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Germany; [2]Institute for Veterinary Epidemiology and Biostatistics, Department of Veterinary Medicine, Freie Universität Berlin, Germany
[3]Department of Physiological Chemistry, Research Center for Emerging Infections and Zoonoses (RIZ), University for Veterinary Medicine Hannover, Germany
[4]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany

*Keywords: standardized methods, minimum inhibitory concentration, routine diagnostics*

**Background and objectives:** In contrast to biocide efficacy testing, biocide susceptibility testing (BST) lacks standardized methods for monitoring pathogens in human and veterinary medicine.
**Materials and methods:** The reference strains Staphylococcus aureus ATCC® 6538, Enterococcus hirae ATCC® 10541, Escherichia coli ATCC® 10536 and Pseudomonas aeruginosa ATCC® 15442 were investigated seven times by broth microdilution for their minimal inhibitory concentrations (MICs) towards benzalkonium chloride, glutardialdehyde, chlorhexidine and isopropanol. All tests were performed using tryptic soy broth as test medium. The following parameters were tested: i) 1st subculture (SC), and 2nd SC, ii) inoculum preparation by direct colony suspension (DCS) with/without glass beads (GB), iii) inoculum density according to the German Veterinary Association (DVG) or the Clinical and Laboratory Standards Institute (CLSI), and iv) incubation at 37°C for 24, 48, and 72 h.
**Results:** Increased incubation times resulted in higher MIC values. Comparing the results for the different times revealed that the highest stability of the values was seen after 24 h. Therefore, the following proposal is made: use of a fresh overnight culture (1st SC or 2nd SC), inoculum preparation via DCS with or without GB, inoculum density according to CLSI or DVG, and incubation at 37°C for 24 h.
**Conclusion:** This method can contribute to the harmonization of BST of bacterial pathogens in routine diagnostics.

# D04

## Autochthonous European histoplasmosis in wild and domesticated animals

Dunja Wilmes, Hendrik Leibhan, Losert Heidemarie, Volker Rickerts

FG16- Mycotic and Parasitic Agents and Mycobacteria, Robert Koch-Institute, Berlin

*Keywords: Histoplasmosis, Europe, molecular epidemiology*

Background: Histoplasmosis is the most prevalent systemic fungal infection caused by an obligat pathogenic fungus worldwide. Genotyping suggests worldwide geographical distribution of this thermally dimorphic fungus. Histoplasma may be divided by molecular typing (multi-locus sequence typing) into several clades identified in Africa, North America, South America, Africa, Asia and Australia. Rare autochthonous human cases and cases in wild and domesticated animals from Europe suggest the presence here as well, but isolation of this fungus from the European environment was achieved only once from soil in Italy
Methods: In order to describe current etiology of histoplasmosis in humans and animals, we first, reviewed the literature, second, analyzed cases diagnosed at the German reference laboratory. Of particular interest were for us, diagnostic methods, presentation and molecular characterization of the Histoplasma strain through multi-locus sequence typing (MLST).
Results: In the literature, we found sixteen published cases of histoplasmosis in wild and domesticated animals in Europe (Germany: n=8; Austria: n=1, Switzerland: n=1; Italy: n=4; Spain: n=1; Denmark: n=1). In our records we found a supplementary eight cases. The infections were described in domestic cats (n=10), badgers (n=8), dogs (n=3) and in one horse, one gazelle and a hedgehog. Most of the infections were diagnosed by PCR and histopathology. The most prevalent presentation of the disease were cutaneous (n=9) and disseminated infections (n=3). In Germany there are seven animal cases which were characterized by MLST and all showed clustering with the Eurasian clade. Our phylogenetic analyses showed a clustering of three human Histoplasma isolates in the same clade. For one of these patients, no travel history was reported.
Conclusion: Our study demonstrates that histoplasmosis is diagnosed in Europe in wild and domesticated animals. These reports may underestimate infections because of unspecific clinical presentation and unspecific histopathology findings. Culturing of a Histoplasma isolate of a European animal infection was not achieved in the last decades, underlining the importance of specific molecular diagnostic tests. Further molecular studies are needed to identify additional loci to increase resolution of MLST or define other typing methods. Environmental sampling is needed to identify Histoplasma in the European environment.

# D05

## Impact of RNA quality on diagnostic sensitivity for BoDV-1 in FFPE-tissues

Arnt Ebinger, Dennis Rubbenstroth, Martin Beer, Dirk Höper

Institute for Diagnostic Virology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health

*Keywords: Borna disease virus 1, formalin-fixed paraffin-embedded, diagnostic sensitivity, RNA-extraction*

Archived formalin-fixed paraffin-embedded (FFPE) brain tissues of presumptive Borna disease virus 1 (BoDV-1) cases of dead-end hosts (incl. humans, horses, sheep) are from particular interest to study its molecular epidemiology and geographical distribution. However, molecular techniques (RT-qPCR, NGS) targeting the extracted RNA are affected by the low RNA quality due to fixation and embedding. Therefore, we analyzed the impact of a longer and a shorter BoDV-1-specific RT-qPCR amplicon assay (P gene: 162 bp, M gene: 75bp) on the diagnostic sensitivity for 88 FFPE brain samples originating from BoDV-1-positive animals and humans from 2004-2018 and compared in detail the RNA-quality level (DV200) with the Cq values for 48 samples. While the 75bp-amplicon detected 96.5% BoDV-1 cases, the diagnostic sensitivity was reduced for the 162bp-amplicon (79.5%). In "low" and "too degraded" RNA-quality levels the Cq values for 162bp-amplicon was significantly higher, whereas there were no differences for "medium" and "high" levels. Based on those results, we evaluated three RNA extraction protocols, in terms of total RNA-yield, DNA content, ß-actin, DV200, and BoDV-1 RNA and developed a superior semi-automated protocol in combination with Covaris truXTRAC FFPE tNA, Agentcourt RNAdvance Tissue Kit, and the KingFisher Flex. This study highlights the significance of selecting an appropriate extraction method and PCR assay for a reliable diagnostic in low-quality samples.

# D06

## Full genome sequencing of West Nile Virus, Europe

J. Schneider[1,2], C. Drosten[1,2,3], V. M. Corman[1,2,3]

[1]Institute of Virology, Charité - Universitätsmedizin Berlin, Berlin, Germany
[2]Dept. of Virology, Labor Berlin, Charité-Vivantes GmbH, Berlin, Germany
[3]German Centre for Infection Research, associated partner Charité, Germany

*Keywords: surveillance, re-emerging virus, encephalitis*

**Background:** West Nile virus (WNV) is restricted to the geographical range of the host. Factors, such as climate change, increased travel and trade, lead to the expansion of this area and thus to a rising number of infections in Europe. In 2018, a high number of human WNV infections were detected in the Mediterranean area, including imported cases to Northern Europe.
**Methods:** For molecular surveillance of imported and autochthonous WNV infections, full genome data are important. Therefore, we developed a high-throughput sequencing (HTS) and a Sanger-based sequencing workflow for full genome sequencing: (I) WNV specific hemi-nested primers amplifying overlapping fragments covering the complete WNV genome; (II) a work frame for unbiased direct HTS sequencing. We applied these two approaches on WNV pos. samples from 5 European countries.

**Results and Conclusions:** The finding of WNV RNA in humans, birds and horses in Germany raised the question of the origin of these infections. The sequence from a human case from Berlin was similar to strains from Italy, belonging to genetic lineage II and was distinct from WNV found in horses and birds in Germany. This is in line with the report that the patient likely acquired the infection during a trip to Italy. Genome sequencing of WNV samples from Eastern European countries resulted in the detection of lineage I and II in 2018 and emphasizes the need for full genome data to track the origin and distribution of WNV infections in Europe.

# D07

## Chlamydial abortion in selected breeding facility of sheep

Ondrejková A., Luptáková L., Petrovová E., Prokeš M., Korytár Ľ., Zemanová S., Čonková-Skybová G.

University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak republic.

*Keywords: Chlamydia, abort, sheep*

Chlamydial abortion is a significant zoonosis associated with reproductive disorders in ruminants. Chlamydial abortion was confirmed in selected breeding facility of sheep. Positive results of CFT for determination of antibodies in sera confirmed the occurence of the disease in the breeding. Out of 37 examined sera 16 were positive, which presents 43.2 % of the total number of investigated samples and 2.28 % of the total sheep number in the breeding. Seroprevalence of antibodies against Chlamydia sp. in ewes was 53.9 % of the total number of 13 examined animals; in rams seropositivity was found in 37.5 % of the 24 examined rams; chlamydia antibodies titer ranged from 1:32 to 1:256. DNA amplification was performed as a standard PCR for the detection of the target region 16S-23S rRNA intergenic spacer. We used primers 16S and 23R previously described by Everett and Andersen (1999) and Everett and colleagues (1999a), which are able to differentiate among nine Chlamydiaceae species. Using these primers we detected DNA of C. abortus in three samples as a template 585 bp long.

This work was supported by the project KEGA 014UVLF-4/2019.

# D08

## pXO2-60, a newly discovered antigen of Bacillus cereus biovar anthracis suitable for seroprevalence analyses

Susann Dupke[1], Constanze Cox[1], Anne Barduhn[1], Emmanuel Couacy-Hymann[2], Fabian Leendertz[1], Chantal Akoua-Koffi[3], Roland Grunow[1], Silke R. Klee[1]

[1]Robert Koch-Institut, Berlin, Germany
[2]LANADA/LCPA, Bingerville, Côte d'Ivoire
[3]Université Alassane Ouattara, Bouaké, Côte d'Ivoire

*Keywords: Bacillus anthracis, Bacillus cereus biovar anthracis, immune response, pXO2-60, serological assay*

Since 2001, atypical B. anthracis-like bacteria, designated as B. cereus biovar anthracis (Bcbva), are known to cause continuous wildlife mortality in a broad range of mammalian species in Taï National Park, Côte d'Ivoire. Furthermore, Bcbva was found in other rainforest areas in Cameroon, Central African Republic, Democratic Republic of Congo and Liberia. The bacteria combine features of B. cereus and B. anthracis (Ba), since they possess both virulence plasmids pXO1 and pXO2 in a chromosomal background of B. cereus.
We identified a protein called pXO2-60, exclusively secreted by Bcbva, but not Ba. Encoded on virulence plasmid pXO2 that also encodes the anthrax capsule, the function of pXO2-60 is still unknown, but the presence of an aerolysin-like domain and a pore-forming activity was hypothesized. Immunization of outbred mice with culture supernatants of Bcbva revealed that pXO2-60 is immunogenic. As expected, antibodies against the protective antigen (PA) were produced by mice after immunization with supernatants of either Bcbva or Ba. No antibodies against pXO2-60 were developed in humans who suffered from classical anthrax, confirming specificity of the antigen.
For retrospective seroprevalence studies in Côte d'Ivoire, we screened sera with PA-ELISA and confirmed results by Western blot stripes coated with PA and pXO2-60. This strategy can be used to investigate the distribution and impact of Bcbva on the human population in countries, where this bacterium is endemic.

# D09

## Safe diagnostics for control of zoonotic pathogens - Development of a new test system for *Coxiella burnetii*

M. Stellfeld[1], S. Henze[2], I.-G. Richter[2], A. Pöcking[2], F. Scholz[3], A. Gütter[3], F. Melzer[1] und K. Mertens-Scholz[1]

[1]Friedrich-Loeffler-Institut, Institut für bakterielle Infektionen und Zoonosen, Jena, Germany
[2]Forschungszentrum für Medizintechnik und Biotechnologie GmbH, Bad Langensalza, Germany
[3]Senova Gesellschaft für Biowissenschaft und Technik mbH, Weimar, Germany

Coxiella burnetii, the etiological agent of Q-fever, causes epidemics in ruminants, particularly in sheep and goats. These local outbreaks are partly linked to human diseases.
The course of Q-fever disease in ruminants is often asymptomatic. In addition, the variable sensitivity and specificity of the serological diagnostics currently used, prevent a rapid and reliable identification of shedding animals. Therefore, new, innovative diagnostic tests are necessary.
The aim of the study is to develop a monoclonal antibody-based pen-side test for the detection of antigens. For this purpose, an infection model based on macrophages and trophoblasts, natural host cells of C. burnetii, will be developed. Transcriptional analysis is used to identify open reading frames (ORFs) that are strongly expressed during infection. In addition, ORFs that are potentially immunogenic according to the literature are cloned and expressed. Their immunogenicity and diagnostic potential will be tested with positive and negative field sera from sheep, cattle and goats.
Monoclonal antibodies against selected proteins will be conjugated with fluorescent particles. These will be used in a membrane-based heterogeneous immunoassay, which will be readable in the barn with a newly developed, mobile scanner.
The development of an affordable, easy-to-use and mobile test system enables the direct and fast detection of infected animals in the field. This test could improve the detection of shedding animals. The elimination of these animals will limit the further spreading of Q-fever and can lead to an improvement in human and animal health together with a reduction in economic and environmental losses.

# D10

## Identification and characterization of Brucella in metagenomic samples

Kerstin Neubert, Knut Reinert

Freie Universitaet Berlin

*Keywords: Metagenomics, Diagnostics, NGS, MLST, Brucella*

Brucellosis is an important worldwide zoonose that severely affects humans and livestock animals. The causative agent Brucella consists of a large group of closely related bacteria. Recent NGS technologies allow analysis of bacteria in metagemomic samples. In the framework of food safety we aim to identify appearance of Brucella in the food chain. Several computational methods are available for taxonomic classification of bacteria at species-level, that are based on alignment of metagenomic reads to reference genomes or exact matches of subsequences of length k (k-mers) to a precomputed k-mer database. Both approaches classify metagenomic reads using the lowest common ancestor (LCA) of matching genomes. To track propagation and control spreading of bacteria analysis on species-level is insufficient. Multi-locus sequence typing (MLST) is a technique in molecular biology that uses fragments of housekeeping genes. Isolates are characterized by a combination of alleles at each loci, which defines their sequence type (ST). For Brucella an extended MLST scheme was developed with 21 independent genetic loci and over 100 known sequence types. We aim to identify further genetic loci based on computational analysis of genome assemblies. Therefore we de novo assembled genomes from a collection of 596 Brucella isolates based on 300 bp paired-end MiSeq reads. We computed the frequency of k-mers in assemblies to identify alleles that distinguish clonal groups or strains.

# Poster Presentations Epidemiology and Ecology of Zoonotic Infections

## E01 – E22

# E01

## One year follow-up of patients with acute Q fever: results from an outbreak in Southwest Germany, 2014-2015

L. Dangel[1, 3],M. M. Meincke[1, 2], C. Wagner-Wiening[1], E. Göhring-Zwacka[1], C. Bauer[4], S. F. Fischer[1, 3]

[1] State Health Office Baden-Württemberg, Stuttgart, Germany
[2] Postgraduate Training for Applied Epidemiology (PAE), Robert Koch Institute, Berlin, Germany
[3] Q Fever Consulting Laboratory, Stuttgart, Germany
[4] Local Health Office, Heidenheim, Germany

*Keywords: Q fever, outbreak, serology, chronic Q fever, fatigue symptoms*

**Background:**
In July 2014, a Q fever (QF) outbreak with over 250 notified cases occurred among visitors of a farmers' market with sheepfold in rural Southwest Germany. The outbreak gave the special opportunity to screen a large number of patients over a long period, who have been infected with one natural point source.

**Methods:**
In this cohort, IgG PhI/II antibodies against Coxiella burnetii were analysed using IFA at defined time points as follow-ups. Twelve months post infection (p.i.) a short fatigue-based-questionnaire was conducted.

**Results:**
Highest IgG PhI/II antibody titers were detected 3 months p.i. and persisted at this level until 9 months p.i.. Still, 12 months p.i. 76.2% of patients revealed high IgG PhII antibody titers. IgG PhI antibody titers were also increased 12 months p.i. in 18% of patients. One patient was identified with chronic QF on the basis of serological and clinical testing. Furthermore a high number (40%) of patients complained of fatigue symptoms 12 months p.i..

**Conclusions:**
Our study clearly demonstrates the importance of long-term antibody surveillance after acute Q fever. At least 3, 6 and 12 months p.i. patients should be monitored for chronic QF. Therefore our study shows that both serology and clinical symptoms should be analysed for the detection of long-term health impacts such as chronic QF and fatigue symptoms. Due to these possible long-term consequences, prevention of Coxiella-infection, such as animal vaccination, is highly recommended.

# E02

## Rabies in animals and humans and post-exposure vaccination in humans in Slovakia – 10 years survey

M. Prokeš, A. Ondrejková, Ľ. Korytár, S. Zemanová, M. Drážovská

Department of Epizootiology and Parasitology; University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

*Keywords: zoonosis, rabies, exposure, prevention, vaccine*

Introduction: Main goals of the rabies prevention programmes may by early detection of disease occurrence and protection of the country against introduction of rabies.
Materials and methods: We analyzed Reports on zoonoses and zoonotic agents and the Annual reports of regional public health authorities in Slovakia in the years 2009 – 2018.
Results: In years 2009 – 2018 were detected 12 cases of rabies in animals in Slovakia. In year 2013 – 7 cases (4x fox, 2x dog, 1x marten) and in year 2015 – 5 cases (5x fox). The risk of infection in man after contact with rabid animal or with animal suspected in rabies: the most cases (1010) were reported in year 2014 with post-exposure vaccination against rabies in 905 cases; minimum cases (819) were in year 2018 and minimum post-exposure vaccination (645) in year 2013. Average in 10 years is 920.3 cases and 787.8 post-exposure vaccinations in humans.
Conclusion: Key activities of rabies prevention are oral vaccination of foxes, vaccination of domestic carnivores against rabies and implementation of surveillance and monitoring programme in whole country, especially focused on the high risk area close to the neighbouring countries with rabies occurrence. One of verified measures is pre-emptive vaccination in animals and humans. Post-exposure vaccination is the special and effective treatment in man, after contact with rabid animal or with animal suspected in rabies.

Acknowledgements: Project APVV-0605-12 and project KEGA 014UVLF-4/2019.

# E03

## Prevalence of Antibodies against Hantaviruses and Leptospira and risk factors for infections of the general population in a Hantavirus high-risk region in Lower Saxony, Germany, 2019

M. Mylius, C. Princk, K. Meyer-Schlinkmann, M. Monazahian, A. Baillot, S. Rettenbacher-Riefler, J. Dreesman

Niedersächsisches Landesgesundheitsamt

*Keywords: zoonoses, seroepidemiologic study, serosurveillance, risk factor, enzyme-linked immuno sorbent assay*

**Introduction:** Hantavirus (H) and Leptospira (L) are notifiable in Germany. Underdiagnoses are likely due to an unspecific clinical course. Risk factors need to be better understood to focus prevention measures. In an ongoing study, we are investigating antibody seroprevalences in residents of a H high-risk region in Lower Saxony.
**Methods:** We test blood samples for IgG-antibodies against H strains and L via ELISA from patients of two surgeries since 2019. Potential risk factors are assessed by a questionnaire. We calculate adjusted odds ratios (aOR) by using logistic regression. Prevalences are calculated with 95% confidence interval (CI).
**Results:** So far, we analysed blood samples of 200 participants (52% male; median age: 61 years), of which 9.5% (95%-CI: 5.8-14.4) tested positive for anti-H IgG and 3% (95%-CI: 1.1-6.4) for anti-L IgG. Four participants had been previously diagnosed with H-disease, none with Leptospirosis. More male than female patients tested positive for anti-H IgG (68% vs. 32%; p=0.15), the percentage of female patients was higher within anti-L IgG-seropositives (83% women vs. 17% men; p=0.08). Using rodenticide was associated with H-seropositivity (aOR: 4.8, 95%-CI: 1.3-17.1).
**Conclusion:** As expected, anti-H IgG was frequently found in our study population. The differences in sex distribution of seropositive participants indicate an association with occupation and leisure time activities. We will present further results on risk factors at the conference.

# E04

## Cross-sectional study on the presence of the zoonotic variegated squirrel bornavirus 1 (VSBV-1) in captive Sciurids in Germany

Valerie Allendorf[1], Dennis Rubbenstroth[2], Kore Schlottau[2], Donata Hoffmann[2], Franz J. Conraths[1], Martin Beer[2], Timo Homeier-Bachmann[1]

[1]Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald-Insel Riems
[2]Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald-Insel Riems

*Keywords: zoonoses, Orthobornavirus, squirrels, VSBV-1, Sciuridae*

The zoonotic variegated squirrel bornavirus 1 (VSBV-1) has so far been found in five species of two subfamilies of Sciuridae. Affected animals were born in captivity and belonged to private and zoological holdings in Europe, primarily in Germany. Sciurids are suspected to serve as reservoir hosts. In humans as accidental hosts, VSBV-1 can lead to severe, often fatal meningoencephalitis. Four cases with lethal outcome, one seropositive survivor and two possible lethal cases have been reported so far.
Objective of this cross-sectional study is estimating the VSBV-1 prevalence in captive Sciurids in Germany. A registry on Sciurid holdings was established to define the total population as the basis for sampling. Due to scarce knowledge on the species-specific susceptibility, the family of Sciuridae was investigated according to the abundance of species in captivity. Diagnostics were performed on buccal swabs and fecal samples by RT-qPCR.
Between 2014 and 2016, 25 VSBV-1-positive squirrels in nine different holdings were identified in Germany. Despite continuous sampling, no additional cases were detected thereafter.
Euthanasia of VSBV-1-positive animals and follow up monitoring of affected holdings likely contributed to the reduction of the incidence. As no comprehensive sampling of Sciurid husbandries has been conducted, single positive animals may have remained undetected. Furthermore, false negative results due to a possible intermittent excretion of viral RNA cannot be excluded.

# E05

## Re-Using existing datasets to analyze possible factors behind the spatial distribution of antimicrobial resistance and hepatitis E virus infection in German wildlife

Taras Günther[1], Marcel Fuhrmann[2], Stephanie Kramer-Schadt[3], Bernd-Alois Tenhagen[2], Reimar Johne[2], Vitaly Belik[1]

[1]Freie Universität zu Berlin
[2]German Federal Institute for Risk Assessment (BfR)
[3]Leibniz Institute for Zoo and Wildlife Research (IZW)

*Keywords: zoonosis, geospatial analyzes, wildlife, hepatitis E virus, antimicrobial resistance*

A continuous increase of human hepatitis E cases in Germany has been observed associated with the consumption of wild boar products. Furthermore, several studies show that antimicrobial resistance (AMR) can be found in wildlife species, which might cause future hazards to public and animal health. Anthropogenic factors, such as wastewater, landfills, and agriculture are assumed to encourage the transmission of resistance to wildlife. However, there is still a lack of knowledge about environmental factors leading to transmission of hepatitis E virus (HEV) and colonization with resistant bacteria in wildlife. In addition, monitoring of wildlife is challenging. This hinders amongst others the development of strategic intervention planning and risk assessment.
Therefore, we will show our first approach to assess and analyze existing datasets of previous investigations in Germany. For the analysis, we use data from different research projects on HEV detection in wild boars from 1995 to 2008 and data on antimicrobial resistance from the German zoonosis monitoring 2016 and 2017 for wild boar (Sus scrofa L.) and roe deer (Capreolus capreolus L.). The datasets will be analyzed with generalized linear mixed models and classification algorithms to assess possible associations between environmental factors and observed cases. We will showcase different approaches to reuse research and monitoring data and discuss how to improve them regarding their reusability and quality.

# E06

## People's knowledge, attitude and preventive practices regarding dengue fever in Central Nepal

Parbati Phuyal[1], Isabelle Kramer[2], David A. Groneberg[1], Meghnath Dhimal[3], Ruth Mueller[4], Mandira Lamichhane Dhimal[5]

[1]Institute of Occupational Medicine, Social Medicine and Environmental Medicine, Goethe University Frankfurt am Main, Germany
[2]PhD student
[3]Nepal Health Research Council, Kathmandu, Nepal
[4]Institute of Tropical Medicine, Antwerp, Belgium
[5]Nepal Pollution Control and Environment Management Centre, Lalitpur, Nepal

*Keywords: Altitude, Chikungunya virus, Dengue virus, Climate change*

**Background:** Dengue fever is rapidly expanding its geographical range globally and also in Nepal.The first reported case of dengue virus infection in Nepal was a Japanese volunteer in 2004, and the first local transmission of dengue virus in Nepal was confirmed during a 2006 outbreak in urban areas of the lowlands. The two most important vectors of dengue virus infections, Aedes aegypti and Aedes albopictus have already established their populations up to 2000 m above sea level in Nepal.
**The aim of this study**: The aim of this study is to assess people's knowledge, attitude on vector borne diseases and their vector prevention and control practices in different altitudinal gradient of central Nepal. This study aimed to evaluate knowledge, attitude, practice (KAP) level of people residing in different geographical regions of Nepal. Furthermore, the main drivers for the practice and social acceptance of different preventive and control measures against mosquitoes are assessed in order to support national one-health strategies to efficiently combat dengue illness in Nepal.
**Methods:** The field survey was conducted in six districts of central Nepal in the month of September and October 2019. A mix method data collection technique was used for data collection, which consists of both qualitative and quantitative methods. Quantitative data was collected by conducting household surveys. We collected information on the socio-demographic characteristics of the participants and their KAP regarding dengue fever using a structured questionnaire. Qualitative data was collected by conducting key-informant interviews and focus group discussion along the altitudinal gradient in central Nepal. We conducted household surveys with 660 research participants, 27 key informant interviews and 12 Focus Group Discussion.
**Findings:** Although the total sampling size was 660, only 651 individuals were interviewed others were non- respondents. Out of 651 interviewed participants, 35.8% were male and 64.2 % were female. Mostly the participants (41.3%) of age group ranging from 30-45 took part in the interview. Most of the people residing in the dengue epidemic areas only heard about dengue. The previously dengue infected people only had complete knowledge about the symptoms of dengue fever, biting behaviour and breeding habitat of dengue vectors neither other people, even the neighbours didn't have knowledge about dengue. However, very few people were only known about dengue vectors. The people from the dengue endemic regions were found to be sensitive about dengue but the people from other districts ever didn't heard about dengue. The knowledge and attitude of people regarding dengue fever vary with the dengue epidemic and non-dengue epidemic areas. The people from dengue endemic areas were found to be more concerned for the prevention and control of dengue. The water service and water storage behaviour influencing the availability of mosquito breeding sites vary with different altitudes. This new knowledge will help to develop necessary components for designing and implementing plans to prevent and control the expansion and impacts of dengue in other dengue non-epidemic areas of Nepal. The data analysis is now on progress and will present complete analysis in the conference.

# E07

## Seroprevalence of *Leptospira spp*. Infections in Cattle from Central and Northern Madagascar

T. Schafbauer[1], A. Dreyfus[2,3], B. Hogan[4], R. Rakotozandrindrainy[5], S. Poppert[2,3], R.K. Straubinger[1]

[1]Institute for Infectious Diseases and Zoonoses, LMU Munich, Germany
[2]Swiss Tropical and Public Health Institute, Basel, Switzerland
[3]Faculty of Medicine, University Basel, Switzerland
[4]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[5]Department of Microbiology and Parasitology, University of Antananarivo, Madagascar

*Keywords: Leptospira; cattle; Madagascar; MAT; seroprevalence*

Background and objectives: Leptospirosis is a bacterial zoonotic disease occurring worldwide, especially in tropical countries. The current prevalence of Leptospira spp. in cattle from central and northern Madagascar is unknown. Hence, the aim of our study was to assess the seroprevalence of pathogenic Leptospira spp. in adult slaughter cattle in these two areas.
Materials and methods: Microscopic Agglutination Test (MAT) with a panel of 12 serovars as antigens was used to test 194 serum samples from zebu cattle. All samples with a titer of ≥1:100 were considered positive.
Results: The overall seroprevalence was 59.3% (95% CI; 52.0-66.2%) with titers ranging from 1:100 to 1:1600. Among all seropositive animals the most frequently antibody reactions were against serovar L. Tarassovi (serogroup L. Tarassovi) with 40.2% (33.3-47.5%), followed by L. Hardjo (L. Sejroe) with 13.9% (9.5-19.8%), L. Grippotyphosa (L. Grippotyphosa) with 9.8% (6.2-15.1%), L. Pomona (L. Pomona) with 7.7% (4.5-12.7%) and L. Autumnalis (L. Autumnalis) with 5.2% (2.6-9.5%). Less than 5% of the samples reacted positive against the remaining seven serovars.
Conclusion: Our results indicate a high exposure to Leptospira spp. of slaughter cattle in central and northern Madagascar. This consequently results in a possible risk contracting this infection and zoonotic disease for people working or living together with cattle.

# E08

## Genotypic characterization of LA-MRSA CC398 in pigsty visitors – temporary or constant colonization?

Natalie Effelsberg[1], Sergej Udarcev[1], Hannah Müller[2], Iris Kobusch[2], Sabrina Linnemann[2], Marc Boelhauve[2], Alexander Mellmann[1]

[1]Institute of Hygiene, University Hospital Münster
[2]South Westphalia University of Applied Sciences

*Keywords: LA-MRSA, epidemiology, WGS, typing*

Background: LA-MRSA CC398 is a rising issue for public health with increasing numbers of severe CC398 infections in humans. Recently it was shown that >75% of sample takers visiting pigsties were at least temporarily colonized with MRSA. However, it was unknown if the visitors were repeatedly colonized during their visits or constantly colonized but temporarily below the detection limit for MRSA. Therefore, we genotyped isolates from repeatedly positive visitors and corresponding pigsties.
Methods: 67 samples from 8 different pigsties and 3 visitors were whole-genome sequenced using Illumina technology. We determined the spa and cgMLST genotypes using the Ridom SeqSphere$^+$ software to analyze their relatedness and examined the phylogeny using the BEAST software.
Results: All 67 isolates belonged to spa-types t011 (n=29), t034 (n=35) or t2011 (n=3) with different types found in the same visitors at different time points. CgMLST analysis revealed 9 clusters mostly correlating with sample site indicating repeated colonization. BEAST analysis confirmed these clusters and suggests an origin of the lineage in the 1960s.
Conclusion: The analyses showed that in most pigsties only few clones were present. The correlation between genotype and sample site suggested repeated de novo colonization of visitors rather than persistent clones.

# E09

## Tick-borne encephalitis (TBE) virus in wild rodents in two natural TBE foci

Hannah M. Schmuck[1], Gehard Dobler[2], Martin Pfeffer[1]

1 Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Germany
2 Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany

*Keywords: TBE, rodents, natural focus*

Tick-borne encephalitis (TBE) is the most important viral tick-borne disease in central Europe. Although well investigated, there still is no valid explanation for the patchy distribution of TBE virus.
One hypothesis is that this focal occurence is result of virus transmission inside rodent burrows by nidicolous tick species. To prove this, we are investigating two well-known TBE virus foci in Bavaria. Every month, we investigate rodents (Myodes glareolus and Apodemus ssp) in a capture, mark, release, recapture study. During serum sampling, ectoparasites are collected from the rodents. Sera are tested for antibodies against TBE and rodents are genetically tested for relatedness to see if the positive rodents are related and living within close vicinity, maybe even in the same burrow. With this project we hope to get (i) a better overview about the seasonal dynamics of rodent infections with TBE virus in a natural setting and (ii) to see whether being positive for TBE is related to some rodent families which in turn would argue for a virus transmission between ticks and rodents (or between rodents) within the burrows.
Thus far, almost 100 rodents have been trapped and the serological results will be presented and discussed. The genetic analysis of the rodents is still pending.

# E10

## What else other than malaria is out there? A systematic review and meta-analysis of acute febrile illnesses in Africa

Martin Wainaina[1,2,3]*, David Attuy Vey da Silva[1], Anne Mayer-Scholl[1], Dirk Hofreuter[1], Uwe Roesler[2], Kristina Roesel[2,3], Johanna Lindahl[3,4,5], Bernard Bett[3], Sascha Al Dahouk[1,6]

[1]German Federal Institute for Risk Assessment, Berlin, Germany
[2]Freie Universität Berlin, Germany
[3]International Livestock Research Institute, Nairobi, Kenya
[4]Swedish University of Agricultural Sciences, Uppsala, Sweden
[5]Uppsala University, Uppsala, Sweden
[6]RWTH Aachen University Hospital, Aachen, Germany

*Keywords: febrile illness, Africa, meta-analysis*

Due to improved malaria surveillance and control programs in Africa over the past decades awareness is currently shifting to non-malarial acute febrile illnesses. Since the burden of febrile diseases remains high, the identification of causative agents is indispensable for adequate treatment and for meaningful infection control measures.
In accordance with PRISMA guidelines, we therefore searched in key databases for epidemiological studies on infectious agents, other than Plasmodium spp., diagnosed in febrile patients in Africa. Data were finally extracted from 74 full text articles reporting on multiple and single etiologic agents. Our ongoing meta-analysis includes qualitative, quantitative and sensitivity analyses.
The dataset comprises 20 African countries mainly located in Eastern (n=39) and Western (n=23) regions, whereas Southern (n=7), Central (n=4) and Northern (n=1) regions are underrepresented. The infections were usually diagnosed by serological tests, such as ELISA (n=21), or culture (n=18) methods. Preliminary results revealed that zoonotic bacteria are a common cause of fever. Studies considering viruses focussed on Dengue (n=11) and Chikungunya (n= 5).
The identification of non-malarial infectious agents contributing to the burden of febrile illnesses on the African continent allows policy makers to make more informed decisions on public health programs and plays a role in preventing indiscriminate use of antimalarial and antibiotic drugs.

# E11

## Forensic investigations of a Q fever outbreak on a German peninsula

B. U. Bauer[1], T. L. Prüfer[2], D. Frangoulidis[3], A. Glenz[1], M. Runge[2], M. Ganter[1]

[1]University of Veterinary Medicine Hannover, Foundation, Clinic for Swine and Small Ruminants, Hannover, Germany
[2]Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Food and Veterinary Institute Braunschweig/Hannover, Hannover, Germany
[3]Bundeswehr Institute of Microbiology, Munich, Germany

*Keywords: Coxiella burnetii, Ruminants, MLVA, phase-specific ELISA*

Background and objectives: C. burnetii was diagnosed as cause of several abortions in a dairy goat farm. Dairy cattle were kept on the same property. The farm is located in an area with a high density of sheep on a German peninsula. Therefore, the study area is exposed to harsh maritime climate condition. Advanced forensic investigations were conducted to identify the origin of the C. burnetii infection and possible spreading to neighbouring sheep husbandries.
Materials and methods: Serum samples and vaginal swabs were examined from 74 dairy goats and 22 dairy cattle. Serum samples were analysed by a phase-specific ELISA and vaginal swabs by qPCR. The genotypes were determined by MLVA/VNTR. Twelve sheep farms located close to the goat farm were visited and vaginal swabs were taken from recently lambed sheep and examined by qPCR.
Results: In dairy cattle, phase-specific ELISA revealed a persistent C.burnetii infection at herd level. Whereas the goats were recently infected with C. burnetii. All vaginal swabs were C. burnetii positive and genotype C1 was identified in both ruminant herds. This points to possible recent transmission of C. burnetii from dairy cattle to the goats. C. burnetii could not be detected in the surrounding sheep flocks. Hence, further spreading of the pathogen did not happen.
Conclusion: C. burnetii transmission between different ruminant species and the influence of local weather conditions should be included in future risk assessments.

# E12

## Investigating potential zoonotic origins of Adenovirus infections in human populations across three different African countries

Antonia Hilbig

Robert Koch Institut / P3 / F.Leendertz

*Keywords: Adenovirus, Africa, Epidemiology, HTS*

Adenoviruses (AdV) are responsible for a wide range of illnesses in a large variety of hosts worldwide. In humans, AdVs represent a severe burden of morbidity and mortality in young children, immunocompromised persons and in the context of hospital acquired infections. Strains associated with severe respiratory disease in humans have been shown to have originated from an animal reservoir, namely wild apes. Cross species detection of AdV in Côte D'Ivoire (CI) has shown "reverse zoonotic" transmission from humans to animals, indicating the potential for domestic animal reservoirs and intermediate hosts. Animal breeding and hunting of bush meat, paired up with poor sanitation, create a strong potential for zoonotic transmissions in rural Africa. To aid in the surveillance of pathogens in low income countries, the African Network for Improved Diagnostics, Epidemiology and Management of Common Infectious Agents (ANDEMIA) was established since 2016. Using the samples and data collected in the first two years of ANDEMIA, we will assess the proportion of patients positive for AdV and their epidemiological characteristics, including contact with wild and domestic animals. In addition, a subset of AdV positive samples collected from Ivorian patients presenting respiratory symptoms will be used for full genome analysis. Discovering the genomic identity of the local AdV species will help us understand their proximity to animal counterparts and hence their zoonotic potential.

# E13

## RoBoPub: Epidemiology of seroprevalences and potential risk factors in forestry workers (FW) in Lower Saxony (LS), Germany, regarding Hantavirus- and Leptospira infections

C. Princk, M. Mylius, K.M. Meyer-Schlinkmann, M. Monazahian, A. Baillot, S. Rettenbacher-Riefler, J. Dreesman

Governmental Institute of Public Health of Lower Saxony, Hanover, Germany

*Keywords: zoonoses, seroepidemiologic study, serosurveillance, risk factor, enzyme-linked immuno sorbent assay*

Background: In Germany, Hantavirus disease and leptospirosis show clinical courses with a wide range of mild to severe symptoms. Due to occupational exposures, the infection rate is probably higher in FW than in the public. We estimated the prevalence, the proportion of undetected infections and associations with potential risk factors in FW. The aim was to formulate targeted prevention messages.
Methods: We analysed blood samples of consenting FW in LS for IgG antibodies against Hantavirus (H) strains and Leptospira ssp. (L) via ELISA. Previous diagnoses and potential risk factors were assessed via questionnaire in 2018. Prevalences were estimated with 95% confidence intervals. Associations of serostatus with risk factors were assessed using uni- and multivariable analysis.
Results: 603 (68%) out of 883 registered FW took part in our study. The median age was 50 years and 90% of the participants were male. Of the 603 blood samples, 54 (9.0%; 95%-CI 6.7-11.2) tested positive for anti-H IgG and 28 (4.6%; 95%-CI 3.0-6.3) for anti-L IgG. Only one of those tested positive for anti-H IgG had been diagnosed with H-disease before, and none for Leptospirosis.
Conclusions: The high seroprevalences and the low rate of diagnoses show that further efforts are necessary to inform risk groups and practitioners on these infections. The high response rate indicates high validity of the results. The analysis of risk factors is ongoing and we will present further results on the conference.

# E14

## How comparable are results from hantavirus seroprevalence studies?

B. Tenner[1], M. Monazahian[2], S. Rettenbacher-Riefler[2], K.M. Meyer-Schlinkmann[2], S. Weiss[1]

[1] Institute of Virology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2] Governmental Institute of Public Health of Lower Saxony, Hanover, Germany

*Keywords: hantavirus, serological assays, Puumala virus*

Hantavirus disease in Germany is caused by Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV). Due to the short viremic phase in patients diagnoses are mainly based on clinical observations and serological tests. There is a variety of commercial and in-house serological assays available but the concordance between these tests is usually not known, hampering meaningful comparisons between different studies. In the framework of a seroprevalence study we investigated the comparability between a commercial assay and our in-house serial testing scheme (ELISA, confirmational Western blot, and immunofluorescence assay). Sera of forestry workers, a risk group for hantavirus exposure, were tested for antibodies against hantaviruses using a commercial IgG ELISA. Positive, equivocal, and a subset of negatively tested sera were subsequently analysed with our in-house scheme for anti-PUUV and anti-DOBV IgG. Out of 877 serum samples 64 (7.3 %) were tested positive and 18 (2.1 %) equivocal using the commercial assay. Preliminary comparison to findings from our in-house scheme shows good concordance for the negative results, but discrepancies for positive samples. We discuss possible reasons for these differences and their impact on conclusions drawn from seroprevalence studies. Our results corroborate the need for detailed test characteristics to allow for meaningful comparison between different studies.

# E15

## Germany-wide analysis of the frequency and the proliferation factors of the zoonotic agent Trichophyton benhamiae

Max Berlin, Yvonne Gräser, Susanne Kosanke, Christiane Kupsch

Charité – Universitätsmedizin Berlin, Nationales Konsiliarlabor für Dermatophyten

*Keywords: Dermatophyte, Trichophyton benhamiae, guinea pig*

Purpose For about 8 years, a new variant of the pathogen T. benhamiae has appeared in Germany, characterized by a previously unobserved culture phenotype with a strong yellow reverse. A few studies suggest that this new variety is now the most common zoophilic dermatophyte in Germany. The guinea pig is very likely the main carrier. Exact prevalence measurements are not yet available. Thus, the aim of our ongoing study is to collect data on the frequency and geographic distribution of the pathogen and its phenotypes (white and yellow) in humans and guinea pigs throughout Germany. Furthermore, animals in breeding farms that are the likely source of transmission and infection are sampled and husbandry conditions are recorded. That puts us in a position to identify propagation factors and to give recommendations for containment.
Methods To analyse the frequency of infections with T. benhamiae, questionnaires were sent to veterinarians, dermatologists and microbiologists. For sampling, the McKenzie brush technique is used. Molecular detection of T. benhamiae and its phenotypes are performed by PCR. Other Trichophyton species are identified by sequencing. Furthermore, we analyse isolates of guinea pigs suspected for dermatophytosis over a several months period in collaboration with the three largest microbiological laboratories of the veterinary medicine.
Results Preliminary data for the year 2018 show, that 72% of the positive guinea pig samples (n=383), were morphologically identified as T. benhamiae. The yellow phenotype was detected in about 90% of the cases by specific PCR.
Conclusion First analyses confirm earlier studies that show that the new (yellow) type of T. benhamiae plays a major role. Since the study is still going on, it will be possible to draw a much more detailed picture at the time of the congress.

# E16

## Rats as potential reservoirs for neglected zoonotic Bartonella species in Flanders, Belgium

Anna Obiegala[1], Maria Krügel[1], Nina Król[1], Christian Imholt[2], Kristof Baert[3], Rainer G. Ulrich[4,5], Martin Pfeffer[1]

[1]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig,Germany
[2]Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Münster, Germany
[3] Research Institute for Nature and Forest, Brussels, Belgium
[4]Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald-Insel Riems, Germany
[5]German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Insel Riems, Germany

*Keywords: flea-borne; Bartonella tribocorum, Rattus norvegicus, Western Europe, Bartonella grahamii*

Background and objectives: Bartonella spp. are zoonotic, arthropod-borne pathogens. Rodents such as Norway rats (Rattus norvegicus) are expected to be reservoir hosts. Nonetheless, the current knowledge of the Bartonella prevalence in rats from Western Europe is scarce. Methods: Rodents were trapped in the context of a rodenticide resistance study in Flanders, Belgium. DNA was extracted from spleen tissue and tested for Bartonella spp. by conventional PCR. Amplicons were sequenced and further analyzed to species level. Results: In total, 1099 rodents were trapped. The predominate species was R. norvegicus (99.64%). Other rodents were: water vole (Arvicola amphibius; 0.18%), fancy rat (R. norvegicus forma domestica; 0.09%) and muskrat (Ondatra zibethicus; 0.09%). PCR analysis resulted in 415 Bartonella DNA positive samples (36.95 %; CI: 34.12-39.85). Bartonella tribocorum (94.62%; CI: 87.90-98.23) was detected most frequently, followed by B. grahamii (3.23%; Cl=0.67-9.14), B. doshiae (1.08%; Cl=0.03-5.91) and an uncultured Bartonella species (1.08%; Cl=0.03-5.91). There was a significant difference in Bartonella prevalence concerning the age of the rats, as well as geographic localisation. However, there was no statistically significant difference in Bartonella prevalence regarding sex, degree of urbanisation and season. Conclusion: Based on the high prevalence found, we conclude that the Norway rat seems to be the key reservoir host for B. tribocorum in Belgium.

# E17

## Pathogenic Leptospira in Small Mammals and Racoons in Lower Saxony, Germany

Martin Runge[1], Jona Freise[2], Silke Braune[1], Maximilian Reuschel[3], Helena Anheyer-Behmenburg[1], Thea Louise Prüfer[1], Enno Luge[4], Cornelia Göllner[4], Anne Mayer-Scholl[4]

[1]Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Food and Veterinary Institute Braunschweig/Hannover, Germany
[2]LAVES, Task Force, Oldenburg, Germany
[3]University of Veterinary Medicine, Foundation, Clinic for small Mammals, Hannover, Germany
[4]Federal Institute for Risk Assessment, Berlin, Germany

*Keywords: Leptospira, muskrats, Norway rats, raccoons, hedgehogs*

In different studies renal samples of muskrats, Norway rats, mice, voles, raccoons and hedgehogs trapped in Lower Saxony, Germany, were investigated for the occurrence of pathogenic Leptospira by a hap1 PCR. Selected positive samples were further investigated by lipl32 PCR, secY sequencing and MLST.
In the first study hap1 was detected in 194 (6.2 %) of 3,155 muskrats and in 125 (21.3 %) of 586 Norway rats. To our knowledge, this is the first investigation of such a large number of muskrats in Germany showing that these are carriers of pathogenic Leptospira.
During the largest German leptospirosis outbreak since the 1960`s in strawberry harvesters in Lower Saxony, 64 mice and shrews were trapped in the affected area in order to identify the source of infection. 46 (71.9 %) mice were hap1 positive. L. kirschneri was detected in 40 of the positive mice. Eight samples showed MLST type (ST) 110, the same sequence type which affected humans. In a follow-up study 519 mice were trapped mainly on the strawberry farms formerly associated with the outbreak. Hap1 was detected in 62 (12 %) mice. Infecting bacteria were identified as L. kirschneri (ST 110), L. borgpetersenii (ST 197, ST 146) and L. interrogans (ST 24).
Interestingly, in a study including racoons, although carriers of Leptospira in North America, only 6 (1.3 %) of 450 animals carried pathogenic Leptospira. Finally, an investigation on hedgehogs showed pathogenic Leptospira in 30 (17.4 %) of 172 animals. Most of them were identified as L. interrogans (ST 24), L. borgpetersenii (ST 146) or L. kirschneri (ST 110).
This work was partly funded by the Federal Ministry of Education and Research (BMBF) under project no. 01KI172H and 01KI1721B as part of the Research Network Zoonotic Infectious Diseases.

# E18

## Case report and tracing the source: A human case of brucellosis caused by Brucella suis biovar 1 in Germany

Enrico Georgi[1], Kim Schneider[2], Holger C. Scholz[3], Markus H. Antwerpen[4], Mathias C. Walter[1], Lothar Zoeller[1], Heiner von Buttlar[5], Johannes P. Borde[6], Sabine Zange[1]

[1]Bundeswehr Institute of Microbiology, D-80937 Munich, Germany
[2]Ortenauklinikum Offenburg-Gengenbach, Gastroenterology and Hepatology, D-77654 Offenburg, Germany
[3]Bundeswehr Institute of Microbiology, D-80937 Munich, Germany
[4]National Consultant Laboratory for Brucellosis, D-80937 Munich, Germany
[5]Bundeswehr Institute of Microbiology, D-80937 Munich, Germany
[6]National Consultant Laboratory for Brucellosis, D-80937 Munich, Germany
[7]Division of Infectious Diseases, Department of Medicine II, University of Freiburg Medical Center and Faculty of Medicine, D-79106 Freiburg i.Br., Germany
[8]Praxis Dr. J. Borde / Gesundheitszentrum Oberkirch, D-77704 Oberkirch, Germany
[9]Bundeswehr Institute of Microbiology, D-80937 Munich, Gerany
[10]National Consultant Laboratory for Brucellosis, D-80937 Munich, Germany

*Keywords: brucellosis, human, Germany*

In July 2018, brucellosis was diagnosed in a German patient without a travel-history to regions endemic for Brucella. Microbiological analysis, including whole genome sequencing, revealed Brucella suis biovar 1 as the etiologic agent. Core-genome based multilocus sequence typing analysis placed the isolate in close proximity to strains originating from Argentina. Notably, despite a strong IgM antibody response, the patient did not develop Brucella-specific IgG antibodies during infection. Here, we describe the clinical course of infection, the extensive epidemiological investigations and discuss possible routes of transmission.

# E19

## "CoxBase" – a new tool for molecular Epidemiology and Surveillance of Q fever

Akinyemi Fasemore[2], Konrad Förstner[3], Thomas Dandekar[4], Andrea Helbich[1], Dimitrios Frangoulidis[1]

[1] Bundeswehr
[2] Bundeswehr/University of Cologne
[3] University of Cologne
[4] University of Würzburg

*Keywords: Q fever, Coxiella burnetii, Typing, molecular epidemiology, surveillance*

The Q fever German Interdisciplinary program for research (Q-GAPS) has committed itself to investigate unsolved questions relating to the epidemiology, immunology, pathogenesis, surveillance and control of Coxiella burnetii, the pathogen of Q fever while striving towards attainment of the "One health" concept. In our sub-project "Genomic and internet based analysis of Coxiella burnetii" we aim at a modern approach of active surveillance, research and joint data integration on the causative agent of Q fever, C. burnetii. Currently we are conducting the integration of strain typing data from C. burnetii into an interactive and publicly available online information platform named "CoxBase" which offers quick retrieval and phylogenetic analysis. Data from newly sequenced strains as well as information about typing results, like tandem repeats suitable for MLVA analysis, which is the gold standard for C. burnetii typing, will be provided. CoxBase is an online platform designed to be capable of presenting metadata with meaningful visual context usable on all existing internet-capable devices. The platform will also provide evolutionary tree drawing functions to aid researches in tracing samples of similar origins as well as spatial links to geographic maps that pinpoint exact location of isolates. CoxBase is being designed to be a valuable tool in the future for all molecular epidemiological and surveillance work and analysis in Q fever.

# E20

## Tick burden on European roe deer as indicator of TBE endemic foci in areas with low TBE incidence in Saxony, Germany

Nina Król[1], Lidia Chitimia–Dobler[2], Gerhard Dobler[2], Yauhen Karliuk[1], Stefan Birka[3], Martin Pfeffer[1]

[1] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany
[2] Bundeswehr Institute of Microbiology, Munich, Germany
[3] Institute of Food Hygiene, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

*Keywords: Ixodes ricinus, roe deer, tick burden, tick-borne encephalitis, Germany*

Background: Saxony is a part of the transition zone between tick-borne encephalitis (TBE) risk areas and those with only sporadic TBE cases. In order to find the missing link between risk and non-risk areas, the aims of this study were: collection and determination of attached ticks and detection of TBE-virus in ticks from roe deer (Capreolus capreolus). Methods: Roe deer coats were provided by hunters from 5 regions: Leipziger Land, Vogtland, Sächsische Schweiz-Osterzgebirge, Nordsachsen and Landkreis Leipzig Borna. Coats were frozen at -80°C, defrosted after few days at 4°C and examined for ticks from both sides, inside and outside. Attached and de-attached ticks were collected and identified under the microscope. Subsequently, ticks were sent to Bundeswehr Institute of Microbiology for TBE virus detection. Results: Out of 134 roe deer, 48 of them were hosts for 1279 ticks. Predominant species was Ixodes ricinus (99.76%; n=1276). Three remaining individuals were identified as Ixodes spp. (0.16%, 1 female and 1 nymph) and Dermacentor reticulatus (0.08%, 1 male). The average infestation was 26.7 (SD=69.5), with the maximal rate of 439 ticks (recorded in May). The dominant life stage of ticks parasitizing roe deer were females (n=536; 42%), followed by nymphs (n=397; n=31.1%), males (n=175; 13.7%) and larvae (n=168; 13.2%). Most collected I. ricinus ticks were de-attached. From 1279 ticks tested, only one (from Sächsische Schweiz-Osterzgebirge) was TBE-positive.

# E21

## Quantitative studies on the development of *Borrelia duttonii* in nymphs and adults of Ornithodoros moubata

M. Schäfer[1], D. Höper[2], C. Silaghi[1]

[1]Institute of Infectology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

*Keywords: tick-borne pathogen, Argasidae, relapsing fever, transmission cycle, flaB*

In recent years, infectious diseases whose pathogens are transmitted by arthropods have become increasingly important in zoonotic research. Ticks, especially the family Ixodidae, are the focus of current research as they are distributed worldwide and transmit numerous viral, bacterial and protozoan pathogens as well as nematodes. Argasid ticks differ in life cycle and feeding pattern from hard ticks. However, they are no less important, as species of the genus Carios, Argas and Ornithodoros play a role as vectors and reservoirs of various zoonotic diseases, including West Nile fever and tick-borne relapsing fever (TBRF) caused by Borrelia spp. spirochaetes.
Our research focused on Ornithodoros moubata, which is a known vector for African swine fever (ASF) and Borrelia duttonii, the primary cause of human tick-borne relapsing fever in the East, Central and Southern Africa. To clarify the infection rates, localization, transstadial and transovarial transmission of B. duttonii in nymphs and adults of O. moubata, we developed a quantitative PCR to elucidate the spirochaete load in coxal fluid, hemolymph, midgut, salivary glands, rectal sac, eggs and after nymphal moult. Initial analyzes showed that the spirochaete load in the midgut of the tick is decreased significantly in the first 48 hours post feeding and not detectable in any other organ up to this point of time. However, the quantification of spirochaete load after 48 hours still needs to be carried out.

# E22

## Acid tolerance of *Coxiella burnetii*

Katharina Sobotta[1*], Bettina Löffler[1], Katharina Boden[1,2]

[1]Institute of Medical Microbiology, Universitätsklinikum Jena, Am Klinikum 1, 07747 Jena, Germany; [2] Dianovis GmbH, Dr. Wichmann-Straße 12, 07973 Greiz

*Keywords: Coxiella burnetii, acid resistance, colony forming units*

**Background:** Q fever is a zoonotic disease caused by intracellular living bacteria Coxiella (C.) burnetii. Currently, the oral infection route by C. burnetii has not been confirmed in humans. Stomach acidic conditions (pH 2-4) are the first defence mechanism to limited food associated infections. In this study, we tested the ability of C. burnetii to survive extreme acid conditions (pH 2-3) to assess the risk of oral infections for human.
**Methods and Results:** We treated C. burnetii with different pH values and calculated the recovery rate by counting colony forming units (CFU). The survival rate of NMII after a 60 min treatment was between 65 % (pH 4) to 1.7 % (pH 2.5) and decreased to a minimum of 0.002 % (pH 2) after 120 min. Treated NMII (pH 2-3.5) develop an acid tolerance between 120 and 180 minutes. The recovery of 8 different C. burnetii strains (pH 2.5, 120 min) resulted in two groups: acid resistant strain with a survival rate between 0.01 and 2.9 % and acid sensible C. burnetii strains.
**Conclusion:** Acid response of C. burnetii is depending on the pH value, time and C. burnetii strains. The data of the study confirm that oral infection of C. burnetii is possible. People with altered gastric acid production like people with Achlorhydria and newborns have a higher risk for oral infection by food contaminated with C. burnetii. Consequently, the digestive infection route of C. burnetii could play a significant role in the transmission of the pathogen.

# Poster Presentations Novel Methods

## M01 – M09

# M01

## Quantitative proteome profiling of Coxiella burnetii reveals major metabolic and stress differences under axenic and cell culture cultivation

J. Dresler[1], J. Klimentová[2], P. Pajer[1], B. Salovska[1], Alena Fucikova[2], Martin Chmel[3], Gernot Schmoock[4], H. Neubauer[4], K. Mertens-Scholz[4]

[1]Military Health Institute, Prague, Czech Republic
[2]Faculty of Military Health Sciences, UoD, Hradec Kralove/Czech Republic
[3]Department of Infectious Diseases, First Faculty of Medicine, Charles University and Military University Hospital Prague, Czech Republic
[4]Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut, Jena, Germany

*Keywords: Q fever; label-free quantification; proteome; oxidative stress; secretion*

Coxiella burnetii, an obligate intracellular bacterium, is the causative agent of the zoonosis Q fever. Classical genetic approaches are not routinely used and transcriptomic data often-missing confirmation on the proteome level. The aim is to use a semi-quantitative proteomics approach to identify isolate specific traits. C. burnetii phase I (NMI) and phase II (NMII) were compared during growth in axenic media with persistently infected mouse fibroblasts. Overall, 659 and 1046 C. burnetii proteins of 2132 annotated coding sequences were identified. Proteome profiles clustered accordingly to the culturing conditions used and indicated different regulation patterns. NMI proteome profiles in axenic media indicate transition from exponential to stationary phase. Regulators such as RpoS, CsrA2, UspA1 and UspA2 were upregulated. Additionally, upregulation of several cell envelope processing, cell division proteins indicate transition from large cell variant to small cell variant. Translocation of proteins via the Sec translocon or type IV B secretion system is present under all conditions. Upregulation of oxidative stress response and transporters associated with osmoregulation was shown. This is the first semi-quantitative analysis of axenically grown with cell culture propagated C. burnetii at the proteomic level. Particularly, transition from exponential to stationary phase and likely adaptations in response to the environment of the parasitophorous vacuole was demonstrated.

# M02

## Ex vivo characterization of neurotropic arboviruses in organotypic mouse brain slices as complementary method for in vivo neurovirulence models

Lars Söder[1,2], Hella Heidtmann[1,2], Christina Puff[3], Wolfgang Baumgärtner[3], Stefanie Becker[1,2], Claudia Schulz[2]

[1]Institute for Parasitology, University of Veterinary Medicine Hanover, Hannover
[2]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hanover, Hannover, [3]Department of Pathology, University of Veterinary Medicine Hanover, Hannover

*Keywords: neurotropic arbovirus, organotypic brain slice culture, complementary methods to animal testing*

Recent emergence and spread of potentially neurotropic arboviruses in Europe, such as Schmallenberg virus (genus Orthobunyavirus) or tick-born encephalitis virus (TBEV, genus Flavivirus), highlight the requirement for methods that allow a rapid risk assessment and characterization of the pathogenesis of novel virus strains in animals and humans. There are a number of reasons to promote complementary methods for animal studies including the reduction of i) experimental animals, ii) financial and time resources, and iii) live animal factors to answer specific questions on a complex cohesive cellular level. For the establishment of an ex vivo method to characterize the neurovirulence of different neurotropic viruses, we compared organotypic brain slice cultures (OtB) from immunocompromised interferon alpha/beta receptor deficient (IFNAR-/-) mice with OtB from immunocompetent C57BL/6 mice. Three different orthobunyaviruses and the flavivirus Langat virus were used as representatives for future studies with related, highly virulent strains such as neglected Ngari orthobunyavirus and TBEV. The replication patterns and peak viral loads were similar (5-10 dpi) in OtB for the different viruses. Considerably higher viral loads over time were generally detected in OtB from IFNAR-/- compared to C57BL/6 mice. In future studies, the tissue integrity and virus distribution will be compared in OtB versus brain samples from animal trials using molecular and morphological techniques.

# M03

## A comparison between Ixodes ricinus nymphs fed in vitro and on calves

Nina Militzer, Sophia Pinecki Socias, Ard M. Nijhof

[1] Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany

*Keywords: Ixodes ricinus, artificial tick feeding, in vitro feeding, vector biology*

Ixodes ricinus is the most common hard tick species in Europe and a vector for many pathogens of veterinary and medical relevance. A promising tool for investigations on tick biology and the infection and transmission of tick-borne pathogens is the artificial tick feeding system (ATFS). To evaluate this method, we compared the feeding parameters for artificial fed nymphs to nymphs fed on cattle. The artificial feeding system consisted of glass tubes with a 20mm diameter closed by an artificial membrane. This small-sized feeding chambers were placed in the wells of a standard 12-well plate, which were filled with 1 mL of bovine blood.
In 2018, 1,990 nymphs were fed artificially using this method and approx. 800 nymphs were fed on cattle as a control group. The mean maximum attachment rate in vitro was 50.4 ± 14%. The engorgement rate of artificially fed nymphs was lower compared to the in vivo control (19 ± 10% vs. 54 ± 4%) and they obtained slightly lower mean weights (3.0 ± 0.9mg vs. 3.5 ± 0.9mg). About 66% of artificially fed nymphs compared to 74% of control nymphs molted successfully, with significantly lower weights for molted females compared to the control group fed on calves (1.29 ± 0.4mg vs. 1.77 ± 0.2mg). In general, the feeding success of I. ricinus nymphs in vitro was lower compared to that of nymphs fed on calves. For the propagation of tick colonies, further optimization of the ATFS will be required for it to become a suitable alternative to the feeding of I. ricinus on experimental animals.

# M04

## Comparison of the detection of Bartonella DNA and other pathogens in paired capillary and venous blood from dogs

Alejandra Álvarez[1], Ricardo Maggi[2], Marta Baxarias[1], Edward Breitschwerd[3], Laia Solano-Gallego[1]

[1] Universidad Autónoma de Barcelona
[2] College of Veterinary Medicine, North Carolina State University (NCSU), Raleigh, North Carolina.
[3] College of Veterinary Medicine, North Carolina State University (NCSU), Raleigh, North Carolina

*Keywords: Bartonella, Mycoplasma, PCR, dogs*

The aim of this study was to determine and compare the presence of Bartonella and other pathogens in paired capillary blood samples and jugular or cephalic venous blood samples from 44 dogs. DNA extraction from blood was followed by Bartonella real-time and conventional PCR. Infection with Ehrlichia/Anaplasma, Hemoplasma and Piroplasms was also investigated. Bartonella apis was identified by real-time PCR and sequenced in venous blood but not in capillary blood. No positive results were obtained for Bartonella by conventional PCR. The proportion of positive results for Hemoplasma in venous and capillary blood was 13.6% and 9.1%, respectively. Positive results and confirmation by sequencing were obtained for M. haemocanis (n=1) and M. haematoparvum (n=5) in venous blood and M. haemocanis (n=1), M. haematoparvum (n=2) and M. fastidiosum (n=1) in capillary blood. Only one dog was PCR-positive for paired venous and capillary blood samples. Results indicate that venous blood appears to be more sensitive for the detection of Bartonella than capillary blood, whereas Hemotropic mycoplasma is identified in both sample types with similar sensitivity. Interestingly, when examining an individual dog, Mycoplasma can be found in either capillary or venous blood samples but not necessarily in both samples. Bartonella apis is reported here for the first time in blood from apparently healthy dogs.

# M05

## New method for detection of zoonozes virus diseases based on surface plasmon resonance and biosensors

Sergey Kravchenko[1], Volodimir Maslov[1], Glib Doroginsky[1], Zinaida Klestova[2], Oksana Blotska[2], Yuliya Dremuh[2], Yuriy Ushenin[1], Alla Yuschenko[2]

[1] V. Lashkaryov Institute of Semiconductor Physics, NAS of Ukraine
[2] State Scientific-Control Institute of Biotechnology and Strains of Microorganisms

There are many methods for viral pathogens detecting. The main for diagnosis is serology and PCR. The most common method is ELISA and immunodiffusion reaction of in agar gel (RID). These methods are relatively long (AGID – 72 hours, ELISA – 2 hours) and sometimes give false-positive results through low sensitivity. Detection of antibodies in infected animal's becomes available for 6–14 weeks. One can use PCR to detect viral genoms, however, needs, time and it is expensive. As new diagnostic method is one based on the phenomenon of surface plasmon resonance (SPR). Compared with the traditional methods, this one has the following weighty advantages: capability to study processes of molecular interaction in nano-sized layers in a real time scale and absence of the necessity to use special markers or fluorescent labels for the studied substan ce. We present that the SPR method enables to detect antibodies to cattle leucosis virus in the diluted solution (1 vol.%) of sick animals semi-positive blood serum, which cannot be made using AGID and ELISA - the serum is considered as negative, the tested animal is considered as healthy, although it is not. Duration of diagnostics by SPR is shorted. Using SPR we detect enteroviruses in distilled and drinking water in the interaction between antigen-antibody. It was shown that using of SPR-method allows detecting antibodies to viruses in lower concentration than the most common methods like ELISA and AGID with the high accuracy.

# M06

## Comparison of the bacterial decontamination efficiency on eggshells via UV-C/UV-C-LED-treatment

K. Rosen[1], Susanne Fleischmann[2], Susann Opherden[2], Thomas Alter[2], Istvan Szabo[3], Sead Hadziabdic, Anette Gensch[4], Peter Rotsch[5], Gero Wiese[6] and Uwe Roesler[1]

[1]Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin, Berlin, Germany
[2]Institute of Food Safety and Food Hygiene, Freie Universität Berlin, Berlin, Germany
[3]German Federal Institute for Risk Assessment (BfR), Berlin, Germany
[4]Löwendorfer Geflügelhof GmbH (LGH), Brandenburg, Germany
[5]OSA Opto Light GmbH, Berlin, Germany
[6]SKS Sondermaschinen- und Fördertechnikvertriebs- GmbH, Berlin, Germany

*Keywords: ultraviolet light, egg, bacteria, contamination, decontamination*

The bacterial contamination of eggshells with food-borne zoonotic agents in the commercial egg production is an important One Health issue. Ultraviolet-C (UV-C) radiation inactivates pathogens and is already used in Germany, inter alia for the water disinfection. A novel system of ultraviolet-C light-emitting diodes (UV-C-LEDs) poses an alternative to the traditionally used UV-C mercury lamps, providing process and efficiency advantages.
The aim of the project is to develop and establish a UV-C LED light method for the commercial application in egg graders. In a first step, the bacterial decontamination efficiency of the UV-C LED light on ESBL-producing Escherichia (E.) coli, Methicillin-resistant Staphylococcus aureus (MRSA), Campylobacter jejuni, and Salmonella on the eggshell surface and conveyer belts will be evaluated and compared to the traditional UV-C light method. Different wavelengths, UV intensities, distances to the treated surfaces and exposure times for the bacterial inactivation by the UV-/UV-LED-treatment will be tested. For this purpose, the surfaces will be inoculated with a defined bacterial concentration of the investigated bacteria and the bacterial count will be estimated before and after the treatment with UV-C/UV-C-LED, respectively. In the second step, the parameters achieving the best inactivation efficiency, will be tested under practical conditions and evaluated for the products´ food safety and quality.

# M07

## Tools to enrich for *Toxoplasma gondii* oocysts from the environment

Benedikt Fabian[1], Bernd Lepenies[2], Gereon Schares[3], Frank Seeber[4]

[1] Robert Koch-Intitute
[2] Tierärztliche Hochschule Hannover
[3] Friedrich-Loeffler-Institute
[4] Robert Koch-Institute

*Keywords: oocysts, nanobodies, One Health*

The apicomplexan parasite T. gondii can infect hosts via two distinct routes. While the ingestion of contaminated meat is assumed to be the main factor of the parasites' transmission to humans, recent findings indicate that T. gondii's environmental stage, the oocyst, must play an important role as infection source for humans, too. Our main aim is to find camelid antibodies (nanobodies) which bind to the outer wall of oocysts in order to isolate and enrich them from environmental samples for subsequent analyses, for example via qPCR.
Since oocysts are only shed by felids including domestic cats, they are generally hard to obtain and thus very precious. Therefore, we first established a method to scan for agents interacting with the outer oocyst wall via immunofluorescence assay (IFA) requiring only minute amounts of oocysts. Next, in order to develop new methods for enrichment of oocysts from the environment we immunised an alpaca with inactivated oocysts to construct a cDNA library that encodes for nanobody sequences. These nanobodies are then screened in phage display and analysed by IFA using oocysts as antigen. Here, we demonstrate the successful immunisation of an alpaca via serological tests and present results from the nanobody library construction.
In addition, we present results from scanning of a C-type lectin receptor Fc-fusion protein library against whole oocysts of T. gondii in order to find more oocyst binding reagents.

# M08

## Distribution of drug induced mutations in HCoV NL63 and 229E analyzed via a newly developed Python3.4- based analysis script

Konstantin Pusl[1], Sebastian Schwinghammer[2], Yue Ma-Lauer[3], Helmut Blum[3], Theresa Graf[4], Franz Cemič[5], Thorsten Hain[5], Albrecht von Brunn[6]

[1] Max von Pettenkofer-Institut, LMU Münc
[2] DZIF-Standort München
[3] Max von Pettenkofer-Institut München, DZIF-Standort München
[4] Laboratory for Functional Genome Analysis, Gene Center, Ludwig-Maximilians-Universität München
[5] IBVA, THM Gießen
[6] Institut für Medizinische Mikrobiologie, JLU Gießen, DZIF Standort -Gießen Marburg Langen-

*Keywords: Virology, Coronaviruses, Mutation mapping, Drug testing, Bioinformatics*

In recent years many new methods were developed in the field of bioinformatics for analyzing Next Generation Sequencing data sets. Software like samtools, bedtools and many others are widely used. However there is a lack of software designed for special applications in the field of Virology. In that context, we are interested in the determination of resistance mutations in whole genomes of coronaviruses after treatment of infected cell cultures with inhibitors directed against cellular cyclophilins. We performed serial passage experiments (up to 25) in the presence of different inhibitors. Viral RNA genomes were extracted from supernatants of infected cells and sequenced using Illumina-NGS. After pre-processing data sets we searched for ways to create distribution maps of mutations occurring in viral ORFs over time upon inhibitor treatment. We developed a Python3.4 based analysis script which is able to use VCF files and VCF summaries for comparing mutations in different samples. Mutations in controls and special types of mutations can be sorted out before the final analysis. The software returns a results file containing a mutations-per-nucleotide ratio and the mean allele frequency for mutations in pre-selected features. The result files can be used for plotting distribution graphs in almost all kinds of graphing software (e.g. GraphPad Prism, R). Especially for R it is possible to implement our script seamlessly into R workflows.

# M09

## Development of a Replicon System for Rabbit Hepatitis E Virus

Filip Cierniak, Rainer G. Ulrich, Martin H. Groschup, Martin Eiden

Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems

Hepatitis E virus (HEV) infects multiple species of animals across the world, including humans. Hosts for the zoonotic genotypes include pigs, wild boar, and rabbits. Experimental infection of rabbits is a promising model system, as infected animals exhibit symptoms similar to those seen during human infection, such as chronic hepatitis and high mortality in pregnant females. Most rabbit HEV sequences segregate into a distinct phylogenetic cluster within HEV genotype 3 and carry a characteristic insertion in open reading frame (ORF) 1, which encodes the nonstructural polyprotein. The underlying molecular mechanisms of infection and pathogenicity remain unknown, as HEV does not replicate well in cell culture. Existing cell culture systems are limited to specific combinations of HEV strains and cell lines. However, insertions in ORF1 can improve in vitro replication of HEV. Therefore, we regard rabbit HEV as a strong candidate for in vitro modelling of HEV infection. Here we present work aimed towards establishing a replicon system based on rabbit HEV. Full-length clones and sub-genomic replicons were used to screen for susceptible cell lines. Initial results showed that further optimization is necessary. Using the optimized system, we aim to assess the effect of insertions and deletions to the HEV genome on virus replication. Finally, we will assess the in vivo infectivity of the resulting virus particles in rabbits and pigs.

# Poster Presentations Pathogen-Cell Interaction

## C01 – C21

# C01

## Mammalian haploid cell screen to identify host factors essential for Rift Valley fever virus

Stephanie Devignot[1], Patrick Schmerer[1], Andreas Leibbrandt[2], Thomas Burkhard[2], Ulrich Elling[2], Josef Penninger[2], Friedemann Weber[1]

[1] Institute for Virology, FB10 – Veterinary Medicine, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
[2] Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), A-1030 Vienna, Austria

*Keywords: Rift Valley Fever Virus, host factors, haploid cells, screening*

Rift Valley Fever Virus (RVFV) is a highly pathogenic arbovirus within the Bunyavirales order (family Phenuiviridae, genus Phlebovirus). It infects mostly domestic ruminants but also humans, and is endemic in Africa. RVFV can cause severe diseases in humans and high abortion rates in livestock, leading to high morbidity and mortality. Hence, Rift Valley fever is named by the WHO as one of eight emerging diseases. The lacking of approved vaccines and therapeutics, and the massive gaps in the understanding of the replication cycle, make it necessary to screen for cellular host factors which are essential for the RVFV cycle.
By using a forward genetic screen we aim at identifying genes and pathways essential for RVFV. A library of haploid mouse embryonic stem cells (ESCs), mutagenized with a revertible genetrap, was infected with the RVFV MP-12 strain. Wild-type ESCs die from infection, while cells mutated in genes essential for the RVFV cycle survive the infection. The mutated phenotype can be reversed by flipping the inserted genetrap with its internal lox site. Sequencing the genetrap insertion sites and bioinformatic analyses lead to the identification of five genes of interest. These genes are currently being validated and under further investigation regarding their involvement in the RVFV replication cycle.

# C02

## Co-infection by swine influenza virus and porcine respiratory coronavirus on porcine differentiated airway cells

Ju-Yi Peng[1], Darsaniya Punyadarsaniya[2], Nai-Huei Wu[1], Dai-Lun Shin[1,] Georg Herrler[1]

[1]Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany
[2]Virology and Immunology Department, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand.

*Keywords: swine influenza virus, porcine respiratory coronavirus, co-infection, air-liquid interface culture system*

Swine influenza A viruses (swIAV) can cause zoonotic infections posing a potential risk to pigs and human health. Among respiratory viruses, swIAV and porcine respiratory coronavirus (PRCoV) are frequently associated in the pig farms. Respiratory co-infections involving various pathogens are far more frequent than single infections, and can cause more severe clinical symptoms. Until now, the role of swIAV and porcine respiratory coronavirus (PRCoV) infections are still underestimated. The molecular interactions of co-infections by swIAV and PRCoV are not well characterized. Here, we aim to understand whether prior swIAV infection facilitates subsequent infection by PRCoV or not. The primary target cells, as well as the main receptor for PRCoV entry, are being studied. To analyze molecular interactions during co-infections, we applied the established air-liquid interface (ALI) culture system for porcine airway epithelial cells. The growth kinetics of the two viruses were determined by focus-forming assay. Also, the expression pattern of surface proteins was characterized by immunofluorescence assay. Infection of porcine airway epithelial cells resulted in the release of infectious virus with a maximum at about 2 dpi. Infection occurred predominantly from the apical side. Comparative staining of viral antigen and α-tubluin indicated that PRCoV preferentially targets non-ciliated cells of the ALI culture system. The ALI culture system will help to elucidate the molecular interactions between pathogens and the host during co-infections.

# C03

## Molecular analysis of the anti-apoptotic effector protein AnkG of Coxiella burnetii

Arne Cordsmeier[1], Max Kraner[2], Walter Schäfer, Utz Fischer[3], Anja Lührmann[1]

[1] Universitätsklinikum Erlangen Mikrobiologisches Institut
[2] FAU Erlangen-Nürnberg Biochemie
[3] Universität Würzburg

*Keywords: Coxiella burnetii, apoptosis, type IV secretion system, effector protein, DDX21*

The obligate intracellular bacterium Coxiella burnetii is the causing agent of the zoonotic disease Q fever. This disease is usually acquired by breathing in dust that has been contaminated by animal feces, urine, milk, or birth products. Q fever is mainly a self-resolving flu-like illness. However, Q fever might develop to an atypical pneumonia, hepatitis or endocarditis.
Inhibition of host cell apoptosis is crucial for C. burnetii to maintain its intracellular niche and, thus, bacterial survival. The anti-apoptotic activity of C. burnetii is mediated by a type IV secretion system (T4SS), which is required to inject effector proteins into the host cell. The T4SS effector protein AnkG is known to exhibit anti-apoptotic properties which depend on its nuclear localization. But, how AnkG alters nuclear function is unclear.
Recently, we identified several host cell proteins involved in RNA metabolism as AnkG binding partners. Here, we focus on the binding of AnkG to one of these proteins, nucleolar RNA helicase 2(DDX21). DDX21 re-localizes into the nucleoplasm after apoptosis induction, which correlates with cell death. Importantly, AnkG prevents DDX21 re-localization and thereby, apoptotic cell death. For the interaction of AnkG with DDX21 the amino acids 1-28 are necessary and sufficient, which is the anti-apoptotic region of AnkG. These results suggest that AnkG alters DDX21 activity and thereby apoptotic cell death.

# C04

## Differences in the interaction of Coxiella burnetii with bovine and human macrophages

Michael Mauermeir[1], Inaya Hayek[1], Norman Müller[2], Christian Menge[2], Anja Lührmann[1]

[1] Institute of Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen
[2] Friedrich-Loeffler-Institut Jena

*Keywords: Coxiella burnetii, Q fever, hypoxia, STAT3, HIF1α*

Coxiella burnetii, the causative agent of Q fever, is an obligate intracellular γ-Proteobacterium. Q fever infection occurs via the inhalation of contaminated aerosols. The manifestation of Q fever depends on host species: in humans, the acute form mainly manifests as a flu-like illness, while the chronic form might develop into a potentially lethal endocarditis. In ruminants like goat, sheep or cattle, Q fever mainly causes reproductive disorders which often result in abortions.
Since the disease manifestation varies greatly between host species, we aim to shed light on differences in the interaction of the pathogen with cells different host species. Macrophages (MΦ) are the primary target of C. burnetii, thus we infected bovine peripheral-blood-derived MΦ and human monocyte-derived MΦ with C. burnetii under hypoxic conditions (0.5% $O_2$) to mimic inflamed tissue.
Our preliminary results suggest that signaling in C. burnetii infected hypoxic bovine MΦ differs significantly from signal transduction in human MΦ. In human MΦ hypoxia leads to HIF1α stabilization, which inhibits STAT3 activation and bacterial replication. In hypoxic bovine MΦ, however, we were able to detect a stable STAT3 activation besides the stabilization of HIF1α. As a consequence bacterial replication was not prevented in bovine MΦ under hypoxia. These results suggest that different signaling in the different host species might result in different pathogenic potential of C. burnetii.

# C05

## Specific circulating microRNAs in acute and chronic hepatitis E can predict chronic hepatitis E

Dominik Harms[1], Bo Wang[2], Mira Choi[3], Kristina Allers[4], Jens Kurreck[5], Jörg Hofmann[6], C.-Thomas Bock[1]

[1] Division of Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Department of Infectious Diseases, Robert Koch Institute
[2] Robert Koch Institute
[3] Medical Department, Division of Nephrology and Internal Intensive Care Medicine, Charité University Medicine
[4] Medical Department, Division of Gastroenterology, Infectiology and Rheumatology (including Nutritional Medicine), Charité University Medicine
[5] Institute of Biotechnology, Technical University of Berlin
[6] Institute of Medical Virology, Charité University Medicine, Berlin, Germany

*Keywords: HEV, acute, chronic, miRNA, liver-specific*

Hepatitis E virus (HEV) infection is a pandemic global health issue with large regional outbreaks. HEV infection is usually self-limiting but can progress to chronic hepatitis E with persisting HEV viremia in immunocompromised individuals depending on the HEV genotypes. Only little is known about the molecular mechanisms involved in different clinical manifestations and transition from acute to chronic hepatitis E. miRNAs are known to regulate viral pathogenesis and are recognized as novel disease biomarkers. Here, we aimed to explore the modulation of serum miRNA signatures in acute versus chronic HEV patients. Viral loads and HEV-genotypes of serum samples from acute (AHE) and chronic HEV-infected patients (CHE) were determined using real-time and nested PCR. HEV RNA titers were in median 3.85E+05 copies/mL in AHE and 2.52E+06 copies/mL in CHE patients, respectively. Phylogeny showed the predominance of HEV genotype 3 in AHE and CHE. Determination of expression levels of liver-specific serum miRNAs was performed using miRCURY LNA real-time PCR. miR-99a-5p, miR-122-5p and miR-125b-5p were upregulated 4.74-, 5.28-, 4.70-fold and 2.69-, 6.34-, 2.28-fold in AHE and CHE patients, respectively, compared to non-HEV controls. Notably, miR-192-5p was increased 2.57-fold in CHE but not in AHE patients. Furthermore, miR-125b-5p is decreased 0.35-fold in CHE patients compared to AHE. Expression analysis additionally showed that let-7a-5p and let-7b-5p are best candidates for endogenous serum reference miRNAs for hepatitis E. To our knowledge, this marks the first investigation concerning the regulation of circulating liver-specific miRNAs in acute and chronic HEV infections. We found that miR-125b-5p and miR-192-5p may be suitable as predictors for chronic hepatitis E. The identified modulated miRNAs may therefore serve as novel biomarkers for the diagnosis, risk assessment and management of hepatitis E.

# C06

## Low NPC1 Receptor Expression Levels in Bat Primary Cells from a Potential Ebola virus Reservoir

M. Bokelmann[1], K. Edenborough[1], P. Kreher[1], U. Vogel[1], A. Lander[1], A. Nitsche[1], H. Feldmann[2], E. Couacy-Hymann[3] and A. Kurth[1]

[1]Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany
[2]Laboratory of Virology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA
[3]Laboratoire National d'Appui au Développement Agricole, Bingerville, Ivory Coast

**Background**
Several studies provide evidence that Mops condylurus, an insectivorous microbat, is a potential reservoir host of ebolaviruses. The significance of the integral membrane protein Niemann-Pick C1 (NPC1) for the entry of ebolaviruses has been determined using cell lines derived from humans, non-human primates and fruit bats. NPC1 receptor expression in the context of ebolavirus replication in microbat cells remain unstudied.

**Material and Methods**
In order to study Ebola virus (EBOV) entry and replication in microbats in vitro, we cultured primary cells from 12 different organs from M. condylurus. The NPC1 receptor expression was characterized by confocal microscopy and flow cytometry. EBOV replication kinetics were studied using qRT-PCR.

**Results**
The NPC1 receptor expression levels in M. condylurus primary cells differed depending on the organ they originated from and was, for most cell types, significantly lower than in human cell lines. In our infection experiments with EBOV, we observed a correlation between NPC1 receptor expression level and virus replication rate in vitro.

**Conclusion**
Our findings unveiled the relationship between NPC1 receptor expression and EBOV replication efficiency in vitro in a potential reservoir species. We hypothesize that low NPC1 receptor expression and virus replication in vitro also correlate with low level virus replication rates in the corresponding tissues in vivo. A divergent organ tropism in the reservoir species could possibly contribute to differences in disease outcome.

# C07

## Genetic modification of bovine intestinal epithelial cells for interaction studies with zoonotic pathogens in 3D-organoids

Soroush Sharbati[1], Jan-Dirk Häger[2], Christian Menge[2], Christian Berens[2]

[1] Freie Universität Berlin, Institute of Veterinary Biochemistry
[2] Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis

*Keywords: Host-pathogen interaction, CRISPR/Cas9, organoids*

Within the framework of the pilot project '3D-KOR' funded by the National Research Platform for Zoonoses, new bovine host-microbiota interaction models are established. The aim of the project is the development of an intestinal 3D cell culture system from bovine colon. To foster the generation of 3D-organoids in vitro, primary intestinal crypt epithelial cells (pCEC) and de-differentiated cell lines (FKD-R) are genetically edited. Immortalisation or re-differentiation is realised by CRISPR/Cas9-mediated genome editing using TERT (telomerase) and miR-147b (Sharbati et al., 2015), respectively.
Both the immortalisation of pCEC and the re-differentiation of FKD-R are achieved by integrating an expression cassette (EC) containing TERT or miR-147b in a 'safe harbour locus' (Wu et al., 2015). It is integrated into the bovine genome by CRISPR/Cas9 and homology directed repair. For the universal generation of the required repair template, we have developed a highly efficient assembly PCR which provides an EC within a few days. ECs produced this way are currently used and suitable clones are characterised.
These models provide a versatile basis for later complex studies dealing with interactions of zoonotic pathogens (e.g. Shiga toxin-producing E. coli, Campylobacter, Shigella or Mycobacteria) or components of the microbiota with the bovine intestine.

# C08

## Proteolytic activation and fusogenicity of human and bat-derived mumps viruses

S Hüttl[1,2], C. Sauder[3], S. Rubin[3], Torsten Steinmetzer[4], Nadine Krüger[1,2]

[1]Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany
[2]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, Hannover, Germany
[3]FDA, CBER, Silver Spring, Maryland, USA
[4]Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marburg, Germany

*Keywords: mumps virus, fusogenicity, proteolytic activation, viral entry process*

The mumps virus (MuV) fusion protein (F) mediates the fusion between viral and host cell membranes as well as between infected and neighboring cells resulting in the formation of multinucleated giant cells (syncytia). It is suggested that the proteolytic activation occurs at a multibasic cleavage motif within the MuV F and is mediated by furin. However, the fusogenicity varies among different MuV strains. Further, it has been shown that the fusogenicity of a bat-derived MuV (batMuV) differs depending on the cell line: Whereas large syncytia were obtained in cells derived from bat species closely related to the host of batMuV, only small/no syncytia have been detected in other mammalian cells. So far, the reasons for the differential fusogenicity as well as the importance for viral replication and pathogenesis are unclear.

Given that all MuVs contain a conserved multibasic cleavage motif, we focused on the 14 amino acids (aa) directly upstream of the fusion peptide. Chimeric F proteins in which these aa were exchanged between human and bat-derived MuVs as well as F proteins and recombinant MuVs harboring point mutations were generated and subjected to further assays.

Interestingly, authentic batMuV and human MuVs harboring batMuV F can replicate in a furin-independent manner suggesting that batMuV F may be cleaved by additional proteases others than furin. Further, the aa at position 8 modifies MuVs fusogenicity without affecting the proteolytic activation.

# C09

## Role of hypoxia in the host-pathogen interaction of zoonotic diseases: Characterization of oxygen level in cerebrospinal fluid during Streptococcus suis meningitis

Nicole de Buhr[1,2], Alexander Martens[1,2], Marita Meurer[1,2], Marta C. Bonilla[1,2], Franz Söbbeler[3], Lara Twele[4], Stephan Neudeck[4], Timo Henneck[1,2], Michael Wendt[5], Sabine Kästner[4], Maren von Köckritz-Blickwede[1,2]

[1]University of Veterinary Medicine Hannover, Department of Physiological Chemistry, Hannover, Germany
[2]University of Veterinary Medicine Hannover, Research Center for Emerging Infections and Zoonoses (RIZ), Hannover, Germany
[3]University of Veterinary Medicine Hannover, Small Animal Clinic, Hannover, Germany
[4]University of Veterinary Medicine Hannover, Clinic for Horses, Hannover, Germany
[5]University of Veterinary Medicine Hannover, Clinic for Swine, Small Ruminants and Forensic Medicine and Ambulatory Service, Hannover, Germany

*Keywords: Streptococcus suis, cerebrospinal fluid, oxygen measurement, hypoxia*

Streptococcus (S.) suis infections can lead to meningitis in humans and pigs. The bacteria enter the cerebrospinal fluid (CSF) by crossing the blood-CSF-barrier (BCSFB), followed by neutrophils as first line of immune defence. This project aims to investigate the host-pathogen interaction in the S. suis infected CSF compartment under pathophysiologically relevant oxygen conditions.
To determine dissolved oxygen concentration in the CSF during infection, fluorescence-based measurement devices were adapted in vivo. Healthy and S. suis-infected pigs were for 7 h during the acute phase of the infection anaesthetised with isoflurane in air/oxygen and physiologic blood oxygenation was maintained. Oxygen concentration, pH, neutrophil numbers and their functionality, bacteria, and DNase activity were determined over time in CSF and/or serum of infected versus control animals. Transmigration of activated neutrophils, formation of neutrophil extracellular traps and subsequent DNase production in infected animals was confirmed.
The detected level of dissolved oxygen in the CSF remained constant independent of the infection status. Thus, transmigrated neutrophils and bacteria did not change the oxygen level in CSF in vivo.
In conclusion, our data enable us to adjust our current 3D cell culture model of the BCSFB to pathophysiologically oxygen values during S. suis infection. Improved in vitro models will help to better understand the host-pathogen interaction for therapeutic intervention.

# C10

## Towards identification of host factors essential for Lassa virus replication

Silke Olschewski[1], Biruhalem Taye Beyene[2], Thorsten Thye[1], Stephan Günther[1], Birgit Förster[1], Maria Rosenthal[1], Jan Kosinski[1]

[1]Bernhard Nocht Institute for Tropical Medicine
[2]Centre for Structural Systems Biology (CSSB) , DESY and European Molecular Biology Laboratory Hamburg

*Keywords: LASV, L protein, AP-MS, spatial proteomics, haploid genetic screen*

Lassa virus (LASV) is the causative agent of the hemorrhagic Lassa fever, which is endemic in West Africa and causes up to 5000 deaths per year. As there is no licensed vaccine and only limited treatment options available, the WHO lists LASV on their R&D Blueprint for urgent development of medical countermeasures. The bisegmented negative-strand RNA virus belongs to the family Arenaviridae and codes for only four viral proteins.
The approximately 250 kDa L protein contains the RNA-dependent RNA polymerase and plays a central role in viral genome replication and transcription. Despite this important function, little is known about interactions between host proteins and the L protein. The aim of our investigations is to identify cellular factors that play a role in viral genome replication and transcription. Therefore, we combine three different screening approaches: (i) affinity purification and mass spectrometry (AP-MS) to identify stable protein-protein interactions, (ii) spatial proteomics (BioID experiments) to identify transient interactions, and (iii) haploid genetic screening to identify cellular pathways essential for LASV replication. We use purified L proteins and also produce three different types of recombinant LASV particles to look at virus-host interactions in the natural context of an infection. The results of the different screening approaches will be combined in an integrated network analysis to select targets for validation and further characterization.

# C11

## The role of C-type lectin receptors in vector/host-virus interactions of arthropod-borne phleboviruses

Kathleen Schön[1,2], João T. Monteiro[1], Dimitri L. Lindenwald[1], Bernd Lepenies[1] and Stefanie C. Becker[2]

[1]University of Veterinary Medicine Hannover, Institute for Parasitology & Research Center for Emerging Infections and Zoonoses (RIZ), Bünteweg 17, 30559 Hannover, Germany
[2]University of Veterinary Medicine Hannover, Immunology Unit & Research Center for Emerging Infections and Zoonoses (RIZ), Bünteweg 17, 30559 Hannover, Germany

*Keywords: Phleboviruses, RVFV, C-Type Lectin Receptors, mosquitos, glycoproteins*

Phleboviruses can be transmitted by arthropods, mainly sandflies, mosquitos and ticks and cause diseases in humans and animals worldwide. The clinical relevance is demonstrated by periodic outbreaks of Rift Valley Fever (RVF) in Africa with severe economic loss due to death in livestock and fatal diseases in humans. Until now, neither vaccines nor specific cures are available for diseases caused by many phleboviruses. Our aim is to understand better the interaction of phleboviruses with the host/vector cells. The superfamily of C-type lectin receptors (CLR) is a large group of carbohydrate-binding pattern recognition receptors of which several members are expressed by antigen-presenting cells.
It was shown that the human CLR DC-SIGN is involved in phlebovirus entry into host cells. We currently use a CLR-hFc library to study the interactions of RVFV Glycoproteins Gn and Gc with CLRs in detail. Those CLR-hFcs are fusion proteins made out of the extracellular part of a CLR and the human-Fc and can be used for binding studies. By using an interspecies approach, we are testing not only human and mouse, but also ruminant and mosquito CLR-hFc fusion proteins. Afterwards, we are studying the direct involvement of binding candidates in phlebovirus entry and replication in a cell-based assay to later perform in vivo experiments. Taken together, the project tackles the question how phleboviruses are recognized by host and vector CLRs and/or exploit CLRs for viral cell entry.

# C12

## *Campylobacter jejuni* genes Cj1492c and Cj1507c are involved in Host Cell Adhesion and Invasion

D. Xi[1], T. Alter[2], R. Einspanier[1], G. Gölz[2], S. Sharbati[1]

[1]Freie Universität Berlin, Institute of Veterinary Biochemistry, Berlin, Germany
[2]Freie Universität Berlin, Institute of Food Safety and Food Hygiene, Berlin, Germany

*Keywords: Campylobacter, pathogenicity, host responses, random mutagenesis*

The pathogenicity factors of Campylobacter jejuni have not yet been adequately clarified. This study aimed to determine further C. jejuni invasion and pathogenicity factors based on a random mutagenesis approach. A mutant library of C. jejuni NCTC 11168 was generated. The motility, adhesion, invasion, cytotoxicity, intracellular survival and cytokine response have been investigated in human intestinal epithelial cells in vitro.
In comparison with wild type, ::Cj1492c and ::Cj1507c showed around 70-80% relative motility and ::Cj1492c had approximately 3-times enhanced adhesion and invasion rates whereas ::Cj1507c possessed clearly diminished adhesive and invasive capability. Only ::Cj1492c had slower in vitro growth rate compared with the parental strain. While 24 h post infection 60% of the intracellular wild type were eradicated, significantly fewer mutants were able to survive. No difference in cytotoxicity and induction of the pro-inflammatory chemokines was determined between both mutants and the wild type.
Multiple factors of C. jejuni may be required to mediate optimal adhesion and invasion of host cells. Here we report two poorly characterized C. jejuni genes with impact on host cell adhesion and invasion in a reverse manner, which seem to have only a limited effect on the host cell response to infection.

# C13

## Quantification of serine protease HtrA molecules secreted by the foodborne pathogen *Campylobacter jejuni*

Matthias Neddermann and Steffen Backert

Friedrich Alexander University Erlangen, Department of Biology, Division of Microbiology, Staudtstrasse 5, D-91058 Erlangen, Germany

Campylobacter jejuni is a major food-borne pathogen and a worldwide health threat. Utilizing different virulence factors, C. jejuni invades the host's intestinal epithelial cell layer. One important factor in this process is the serine protease HtrA, which is secreted into the extracellular space, and helps the bacteria to transmigrate across the gut epithelium by cleaving various cell-cell adhesion proteins. The aim of the present study is to quantify the amount of HtrA molecules secreted per bacterial cell in liquid culture and during infection.
HtrA protein purification and quantitative Western blotting were used to determine the number of HtrA molecules secreted by two C. jejuni model strains, 11168 and 81-176, in liquid culture during an 8-hour time course. On average, the two strains yielded similar HtrA secretion rates, with strain 11168 secreting 4,314 ± 949 molecules and 81-176 secreting 5,483 ± 1,246 per bacterium after 2 hours. After 8 hours, both strains showed a decrease in the average amount of HtrA secreted per bacterial cell over time. Secretion of HtrA by strain 11168 reduced to about 1,772 ± 520 molecules and only 2,151 ± 562 HtrA molecules were secreted by strain 81-176 at this time point. During infection of gut epithelial cells, the secretion of HtrA is slightly higher with a similar secretion pattern over time compared to culturing in vitro. Taken together, we determined the number of HtrA molecules secreted by single C. jejuni cells over time. The results suggest that HtrA secretion is regulated in a time-dependent fashion, leading to increasing accumulative HtrA concentrations in the extracellular medium.

# C14

## Peptidase PepP is a novel virulence factor of Campylobacter jejuni contributing to murine campylobacteriosis

Markus M. Heimesaat[1], Anna-Maria Schmidt[1], Soraya Mousavi[1], Ulrike Escher[1], Nicole Tegtmeyer[2], Daniel Simson[2], Silja Wessler[3], Gabriele Gadermaier[3], Peter Briza[3], Dirk Hofreuter[4], Stefan Bereswill[1], and Steffen Backert[2]

[1]Institute of Microbiology, Infectious Diseases and Immunology, Gastrointestinal Microbiology Research Group, Charité - University Medicine Berlin,
[2]Division of Microbiology, Department of Biology, Friedrich Alexander University Erlangen/Nuremberg, Erlangen, Germany
[3] Department of Biosciences, Paris Lodron University of Salzburg, Salzburg, Austria;
[4]Department of Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

The underlying mechanisms of host-pathogen interactions resulting in immunopathological responses upon human Campylobacter jejuni infection are not completely understood, but the recent availability of murine infection models in microbiota-depleted IL-$10^{-/-}$ mice mimicking key features of campylobacteriosis helps solving this dilemma. Here, we have used gnotobiotic IL-$10^{-/-}$ mice as a model system to characterize the function of a novel virulence factor of C. jejuni. We have applied growth techniques, casein zymography, RT-PCR, ELISA and immunohistochemistry to determine important infection parameters by the bacteria. During a screen for proteases expressed by C. jejuni, we identified a peptidase of the M24 family as a potential novel virulence factor, which we named PepP. The gene is strongly conserved in various Campylobacter species. A constructed deletion mutant ΔpepP of C. jejuni strain 81-176 grew as efficiently as isogenic wildtype (WT) bacteria on rich media. To shed light on the potential role of this protease in mediating C. jejuni-induced immunopathological responses in the mammalian host, we perorally challenged microbiota-depleted IL-$10^{-/-}$ mice either with the WT strain or the ΔpepP deletion mutant. Upon infection, both strains could stably colonize the murine gastrointestinal tract with comparably high loads. Remarkably, pepP deficiency was associated with less severe induced malaise, associated with less distinct apoptotic and innate immune cell responses, but also with more pronounced proliferative/regenerative epithelial cell responses in the large intestines six days post-infection. Furthermore, pro-inflammatory mediators were lower in the colon, ileum and mesenteric lymph nodes of mice that had been challenged with the ΔpepP mutant compared to those infected with the WT strain. This also held true for extra-intestinal organs including liver, kidneys and lungs, and, strikingly, to systemic compartments. Taken together, our study provides first evidence that the protease PepP of the M24 family is a virulence determinant involved in mediating campylobacteriosis in the mammalian host. The finding that apoptosis in the colon is significantly diminished in mice infected with the pepP mutant highlights the epithelial layer as the first and main target of C. jejuni PepP in the intestines.

# C15

## Determination of oxygen concentration in vivo and in cell culture research during infection by fluorescence-based measurement technique

Alexander Martens[1,2], Katja Branitzki-Heinemann[1,2], Nicole de Buhr[1,2], Maren von Köckritz-Blickwede[1,2]

[1]University of Veterinary Medicine Hannover, Department of Physiological Chemistry, Hannover, Germany
[2]University of Veterinary Medicine Hannover, Research Center for Emerging Infections and Zoonoses (RIZ), Hannover, Germany

*Keywords: Oxygen measurement, cell culture research, in vivo, fluorescence, hypoxia*

To better characterize the host-pathogen interaction of zoonotic infections, optimized cell culture systems are needed. The adaptation of in vivo relevant oxygen conditions is essential, since oxygen affects the behaviour of several cell types during host-pathogen interactions. Most host cells are commonly cultivated under atmospheric oxygen concentrations, although the physiological oxygen conditions in vivo are significantly lower in most tissues. Especially during an acute infection, oxygen concentration locally decreases to hypoxic conditions. Very little data of in vivo pathophysiological oxygen concentration during infections at the site of infection are known. The goal was to establish the detection of oxygen concentration in vivo and in vitro in cell culture research during infection using oxygen-sensitive fluorescence probes.
Here, we measure oxygen concentrations in cell cultures with the SDR Sensor Dish Reader / Oxy-1 ST + Sensor spot, PreSens GmbH, Germany. Suspension cells as primary blood-derived neutrophils or bone-marrow-derived mast cells show higher oxygen concentrations compared to adherent epithelial cells grown in a monolayer. Thus, the oxygen concentration depends on the cell culture system used. Furthermore, we detected in vivo oxyen concentrations in cerebrospinal fluid of living healthy and infected pigs using a similar approach to be able to compare in vitro and in vivo results. Measurements in various solid tissues are currently optimized.

# C16

## Comparative analysis of host cell entry driven by glycoprotein complexes of diverse lymphocytic choriomeningitis virus (LCMV) variants

Berenice Fischer, Markus Hoffmann, Stefan Pöhlmann

*Keywords: LCMV, host cell entry, organ tropism, alpha-dystroglycan, SKI-I/S1P*

**Background and objectives**
Lymphocytic choriomeningitis virus (LCMV) has its reservoir in rodents. It can be transmitted to humans via inhalation of aerosolized particles or ingestion of contaminated food, and can induce disease ranging from flu-like symptomatic to meningitis. Recently, clusters LCMV-related deaths occurred in organ transplant recipients. Moreover, infection of pregnant women can lead to death of the unborn child. Cellular entry is mediated by the viral glycoprotein complex (GPC) that facilitates attachment and membrane fusion. Within this study we sought to investigate whether diverse LCMV variants differ in their zoonotic potential.

**Materials and methods**
We used transduction vectors to study GPC-driven cellular entry. Further, we investigated GPC-priming by SKI-I/S1P and analyzed whether alpha-dystroglycan (aDG) or additional attachment factors are equally utilized by GPCs from all tested LCMV variants.

**Results**
GPC-driven entry into kidney- and lung-derived cell lines was high for all LCMV variants, whereas liver cells showed attenuated entry for variants found in wood mice and during lethal LCMV outbreaks among captive marmosets. GPC-priming by SKI-I/S1P and endosomal low pH were equally required for all LCMV variants. Finally, ectopic aDG, DC SIGN and TIM 1 expression augmented GPC-driven entry.

**Conclusion**
We identified variant-specific differences in the efficiency of GPC-driven host cell entry and defined cellular factors that generally support LCMV entry.

# C17

## HCoV Papain-like Proteases Target Ring Domain Containing E3 Ubiquitin Ligases

Y. Ma-Lauer[1,2], Z. Yu[1,2], B. von Brunn[1,2], D. Niemeyer[3], C. Drosten[3], A. von Brunn[1,2]

[1,2]Max von Pettenkofer-Institut, LMU München;
[2]DZIF, Standort München
[3]Charité Universitätsmedizin Berlin, Institut für Virologie

Most human coronaviruses encode two papain-like proteases (PLP1 and PLP2) whereas highly virulent SARS-CoV and MERS-CoV only encode one papain-like protease (PLpro) in nonstructural protein 3. Ring finger and CHY zinc finger containing protein 1 (RCHY1) is an E3 ubiquitin ligase and we had identified it as an interacting partner of SARS-CoV PLpro, MERS-CoV PLpro, and HCoV NL63 PLP1 and PLP2 via split-YFP as well as co-immunoprecipitation. All of the tested viral papain-like proteases dramatically stabilized RCHY1 and lead to strongly increased polyubiquitination and degradation of anti-viral factor p53 as a substrate of RCHY1.
We now identified E3 ubiquitin ligases makorin ring finger protein 2 and 3 (MKRN2 and MKRN3) as targets of HCoV papain-like proteases. SARS-CoV PLpro, MERS-CoV PLpro and NL63 PLP2 cause strong accumulation of MKRN2 at the protein level whereas NL63 PLP1 does not. Interestingly, only SARS-CoV PLpro and NL63 PLP2 strengthen MRKN2 signaling to further suppress NF-ƙB –mediated cytokine expression. However, MERS-CoV PLpro and NL63 PLP1 suppress NF-ƙB –mediated cytokine expression in an MKRN2-independent manner. E3 ubiquitin ligase MKRN3 is stabilized by HCoV papain-like proteases as well and it is especially receptive to MERS-CoV PLpro. Using MERS-CoV PLpro as bait, we screen a human E3 ubiquitin ligase library by yeast-two-hybrid assays to identify more E3 ligase candidates targeted by HCoV papain-like proteases. E3 ubiquitin ligases seem to play important roles for CoV replication. We therefore consider them as important cellular checkpoint targets for antiviral intervention.

# C18

## Chikungunya Virus Pathology and Antiviral Responses in a Highly Relevant Primary Cell Model

Fabian Pott, Richard Brown, Thomas Pietschmann, Elena Neumann, Christine Goffinet

*Keywords: Chikungunya, Fibroblasts, Innate Immunity, Interferons, Primary Cells*

Chikungunya virus (CHIKV), a mosquito-borne alphavirus, has gained increasing attention in recent years due to several outbreaks in its endemic areas and in the western world. The clinical symptoms include fever and a characteristic polyarthralgia, which can persist over months to years. Here we present our findings regarding the interplay between CHIKV and the cell-intrinsic and induced innate immune reactions in selected cell lines and a primary human synovial fibroblast model. We find synovial fibroblasts to be a functional, easily accessible model system that supports the full replication cycle of CHIKV. Interestingly, synovial fibroblasts are a putative site of CHIKV low-level replication and persistence and may contribute to the arthritis-like ailments suffered during and after CHIKV infection in vivo. Upon challenge with CHIKV, synovial fibroblasts strongly induce transcription of numerous interferons (IFNs) and interferon stimulated genes (ISGs) as well as proinflammatory cytokines, which in turn leads to upregulation of antiviral proteins. Flow cytometric analysis reveals non- or abortively infected bystander cells as the major source of the antiviral mediators, while the immune response in the productively infected cells appears to be suppressed by the virus. Additionally, pre- and poststimulation with IFN type I and III lowers the infection rate by up to 90% in these cells, with type III IFN inducing milder but still efficiently restricting ISG responses.

# C19

## Studying cellular barriers for zoonotic respiratory viruses at the protein level

Z. Yu, Y. Ma-Lauer, B. von Brunn, A. von Brunn

Max von Pettenkofer-Institut, LMU München and DZIF, Standort München

*Keywords: Cellular barriers for virus infection, virus-host protein-protein interaction, Y2H, cellular checkpoint genes*

Zoonotic respiratory viruses like MERS-CoV can cross species barriers and replicate in different hosts with no or little pathogenicity in animal reservoirs, but causing serious illness in humans. To understand barriers and pathogenicity it is important to gain knowledge on the function of the individual viral proteins in animal and human host cells, on their interactions with viral and cellular proteins and on the consequences of these interactions on cellular signaling pathways. Great knowledge has accumulated in the literature on the interplay of cellular with proteins of various viral species allowing the prediction of cellular pathways required for viral replication. We utilize this knowledge to construct a new, quickly mobilizable Yeast-2-Hybrid (Y2H) - based, defined human cDNA library reduced to most important cellular signaling pathways and target genes known to be involved in viral replication. We report on the construction of a Y2H-based library containing human checkpoint genes including interferon-stimulated genes, ubiquitinases etc. As viral model for testing protein interactions we use the MERS-CoV orfeome.

# C20

## Investigations into coinfections of the obligate intracellular ruminant pathogens Chlamydia abortus and Coxiella burnetii

Sara Balk, Elisabeth Liebler-Tenorio, Christiane Schnee

Friedrich-Loeffler-Institut, Institut für molekulare Pathogenese

Chlamydia abortus and Coxiella burnetii are obligate intracellular gram negative bacteria that infect small ruminants.Both target the placenta, can cause abortion and possess a zoonoticpotential. Chlamydia and Coxiella share even more striking similarities onthe cellular and molecular level such as a biphasic life-cycle withextracellular, infectious variants and intracellular, non-infectious formsresiding in a membrane-bound vacuole. By hijacking intracellular organellesand redirecting transport vesicles, the bacteria acquire essential nutrients,but in a different mode. While the vacuole of Coxiella is an acidifiedphagolysosome which fuses with endocytic vesicles, Chlamydia in non-acidifiedinclusions receives its nutrients from fusiogenic events with exocyticvesicles from the Golgi apparatus and the endoplasmatic reticulum. Fieldstudies in small ruminants have shown coinfections of Chlamydia and Coxiellain placental tissue from abortions. We have screened 65 placenta samplescollected after normal parturition from infected sheep flocks. 52.3 % of
these samples were PCR-positive for Chl. abortus, 61.5 % for Cox. burnetiiand in 40.0 %, a coinfection of both agents was detected. To investigate whether the interaction of the two pathogens is of synergistic, competitive
or neutral nature and to better assess the contribution of such polymicrobial infections to disease progression, we analyzed the interaction of Chl. abortus DC59 and Cox. burnetii RSA 439 NMII in cell culture models.
Fluorescence and electron microscopy revealed that different cell lines can be coinfected with Coxiella and Chlamydia and that a single cell can harbour both pathogens. They reside in distinct vacuoles but in close proximity to each other with occasional fusion of vacuole membranes. A preinfection of cells with Coxiella does not alter general Chlamydia morphology, but growthand infectivity was negatively influenced as shown by qPCR analysis of DNAreplication and titration.

# C21

## Bacillus cereus biovar anthracis – insights into virulence and gene regulation

Silke R. Klee[1], Susann Dupke[1], Constanze Cox[1], Kym Antonation[2], Pierre Goossens[3], Roland Grunow[1], Chantal Akoua-Koffi[4], Emmanuel Couacy-Hymann[5], Fabian H. Leendertz[1]

[1]Robert Koch-Institut, Berlin, Germany
[2]Public Health Agency of Canada, Winnipeg, Canada
[3]Institut Pasteur, Paris, France
[4]Université Alassane Ouattara, Bouaké, Côte d'Ivoire
[5]LANADA/LCPA, Bingerville, Côte d'Ivoire

*Keywords: Bacillus cereus biovar anthracis, virulence, plasmids, gene regulation*

Bacillus cereus biovar anthracis (Bcbva) was first detected in 2001 in chimpanzees that had died of an anthrax-like disease in the Taï National Park (TNP) in Côte d'Ivoire. Similar bacteria were isolated later in other African rain forest areas. These bacteria form a distinct clade in the B. cereus group which is separate from the monomorphic clade of classic Bacillus anthracis (Ba). Bcbva possesses two virulence plasmids responsible for toxin and capsule production, but its chromosomal background points to a non-Ba member of the B. cereus group. Therefore, detection based on bacteriological features is impeded. Regulation of virulence genes is similar both in Ba and Bcbva and depending on the regulator AtxA. Expression of toxin and capsule genes is upregulated under host mimicking conditions ($CO_2$ atmosphere). We currently perform comparative RNA sequencing for a deeper insight into the global gene regulation of Ba and Bcbva. Virulence of Ba and Bcbva determined by $LD_{50}$ is comparable in small animal models, but Bcbva is able to synthesize a second type of capsule composed of hyaluronic acid, which might play a further role in pathogenicity. No human cases of anthrax caused Bcbva were reported until now. In a DFG-funded German-African cooperation project, we performed seroprevalence studies using a Bcbva-specific antigen and confirmed human exposition, most likely due to hunting and consumption of bush meat.

# Poster Presentations Antimicrobial use and resistance

## A01 – A27

# A01

## Characterization of *Staphylococcus aureus* isolates from naked mole rats

Henrike Krüger[1], Kristin Mühldorfer[2], Ralf Ehricht[3,4], Stefan Monecke[3,4,5], Stefan Schwarz[1], Andrea T. Feßler[1]

[1]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[2]Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Berlin, Germany
[3]Optical-Molecular Diagnostics and System Technology, Leibniz Institute of Photonic Technology
[4] InfectoGnostics research campus, Jena, Germany
[5] Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany

*Keywords: spa typing, antimicrobial susceptibility testing, broth microdilution, penicillin resistance, tetracycline resistance*

**Background and objectives:** Staphylococcus aureus can colonize humans and animals and plays an important role as zoonotic pathogen. During recent years, numerous studies focused on staphylococci from humans and animals including zoo and wildlife species. The aim of the present study was to investigate the resistance properties of S. aureus isolates from naked mole rats (Heterocephalus glaber) kept in captivity.
**Material and methods:** In total, eight S. aureus isolates from five individuals were cultured from bite wound abscesses. They were investigated for their enzymatic properties and identified by MALDI-TOF MS and spa typing. Antimicrobial susceptibility testing for 31 antimicrobial agents was performed by broth microdilution. Specific resistance genes were investigated by PCR.
**Results:** All isolates were coagulase and hyaluronidase positive. Two spa types t6544 (n=5) and t7200 (n=3) were detected, differing in a duplication of r12 in t7200. Susceptibility testing revealed that all isolates were resistant to penicillin and ampicillin and harbored the β-lactamase gene blaZ. The three isolates with spa type t7200 were additionally resistant to tetracycline. Two isolates were positive for the tet(K) gene, while the remaining isolate harbored the tet(L) gene.
**Conclusion:** The isolates from naked mole rats displayed two related spa types and only limited resistance properties. This is a favorable situation regarding a possible zoonotic transmission between animals and humans.

# A02

## Population-wide determination of antibiotic resistance in clinical isolates of Listeria monocytogenes from Germany

Martin A. Fischer, Sven Halbedel

Robert Koch-Institute, German-Austrian Binational Consiliary Laboratory for Listeria, FG11 - Enteropathogenic Bacteria and Legionella, Wernigerode, Germany

*Keywords: Listeriosis, gastrointestinal zoonotic infection, antibiotic resistance, microdilution assay*

Listeria monocytogenes is an important food-born pathogen mostly associated with milk products, but also occurring on meat and vegetables. As a zoonotic pathogen it is responsible for listeriosis, an illness ranging in its symptoms from gastroenteritis to septicemia, meningoencephalitis and abortion in pregnant females. Case numbers are increasing during the last years with almost 700 notified cases in 2018 in Germany. Even though L. monocytogenes is susceptible to a variety of antibiotics, fatality rates range between 7 to 30 %. Therefore, listeriosis is one of the most fatal gastrointestinal zoonotic infections.
Monitoring the development of antibiotic resistance is of utmost importance. Within the framework or the German consiliary laboratory for listeriosis over 500 selected human isolates from all phylogenetic clades are currently screened for their antibiotic susceptibility towards 14 clinically relevant antibiotics.
All isolate were sensitive against penicillin and gentamycin and were resistant towards ceftriaxone. In addition, resistance towards daptomycin and ciprofloxacin as well as intermediate susceptibility towards tetracycline and linezolid was frequently observed. These findings could be an indication for an ongoing development of multidrug resistance. In addition, knowledge of frequent co-occurrence of certain antibiotic resistances might be valuable information during treatment. Current results of this ongoing research will be presented.

# A03

## Zoonotic multidrug-resistant microorganisms among healthy pigs in Germany

Andreas Schlattmann, Knut von Lützau, Ursula Kaspar, Karsten Becker

University Hospital Münster

*Keywords: Staphylococci, macrococci, resistance, livestock, pigs, mec genes*

Livestock-associated methicillin-resistant (MR) Staphylococcus aureus (LA-MRSA) colonize porcine nasal cavities and pose a threat to human health care. Known genes conferring methicillin resistance in members of the Staphylococcaceae family are mecA, mecB, mecC, and mecD.
We investigated intranasal and snout surface swab samples taken from 27 pigs being kept on 7 different farms. From these, 51 S. aureus strains were isolated, spa typed, and screened for methicillin resistance. Each farm harbored at least one pig with at least one S. aureus isolate. The spa types were t889 (n=15), t337 (n=10), t8893 (n=6), t034 (n=5), t011 (n=4), t12359 and t17059 (n=3), t1298 and t1419 (n=2), and t2315 (n=1). Methicillin resistance was detected in 25 isolates (49%) with 15 isolates positive for mecA and 10 negative for all known mec genes. On 6 out of 7 farms, 209 non-S. aureus staphylococci have been found with 33 isolates (15.8%) exhibiting phenotypic methicillin resistance. Six isolates of these MR isolates were negative for all mec genes. Furthermore, we isolated two ”Macrococcus goetzii” strains that tested positive for mecB.
In conclusion, we have found staphylococci on all farms with simultaneous colonization of methicillin-susceptible and MR isolates. This indicates that even without direct selective pressure, methicillin resistance is detectable in a subgroup of staphylococci. The lack of known resistance determinants in some MR isolates warrants future investigation.

# A04

## Towards an improved microbiological detection method for carbapenemase-producing Enterobacteriaceae

N. Pauly[1], J.A. Hammerl[1], M. Grobbel[1], B.A. Tenhagen[1], A. Käsbohrer[1, 2] and A. Irrgang[1]

[1]German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany
[2]Institute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

*Keywords: Carbapenemase, plasmid, tenacity, detection method*

A reliable method for highly sensitive detection of carbapenemase-producing Enterobacteriaceae (CPE) in animals and food products of animal origin is essential to assess the prevalence of CPE in livestock. The current protocol as part of the EU-wide monitoring in animal and environmental samples is unsatisfying for detection of CPE in samples with low concentration. With first experiments, the One Health European Joint Program project IMPART works towards the goal of method improvement.
To optimize this method, initial experiments were carried out to determine the tenacity of CPE in feces and food matrices of animal origin. Therefore, the CFU of seven different CPE (four E. coli and three Salmonella spp.) in eight matrices (poultry feces, pig feces, bovine feces, milk, fish, poultry meat, pig meat and bovine meat) were determined microbiological over 10 days. Furthermore, the stability of plasmids in CPE was determined by controlling the CFU with and without selection, by PFGE, and occasional by sequencing.
The experiments showed that CPE survive stable in food products but not in feces, where they decreased 2 log levels during the first 24 h. The stability of resistances on the plasmids was shown and confirmed after more than 500 generations.
These results indicate that it could be useful to prepare feces samples by buffering. In conclusion, initial changes have improved the detection method. Further parameters will be tested and optimized.

# A05

## Dissection of a New qnrD2 M. morganii Plasmid Isolated from Systematically Diseased Cold-Blooded Amphibians

K. Juraschek, C. Jäckel, C. Banyai, S. Schmoger, T. Skladnikiewicz-Ziemer, B. Lesniewsky, K. Nöckler, J. Beutlich, A. Käsbohrer and J.A. Hammerl

German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany

*Keywords: Quinolone, PMQR, qnrD*

Fluoro(quinolones) are important antimicrobials for treatment of animal and human infections. Several plasmid-mediated resistance factors were reported to be involved in the occurrence of quinolone resistant bacteria. This study was initiated to compare stability, antimicrobial susceptibility and the genetic background of qnrD plasmids from M. morganii comprising different pentapeptide repeat protein D subclones. Bacteriological investigation of systemic diseased African bullfrogs (P. edulis), resulted in the isolation of four M. morganii isolates exhibiting reduced susceptibility to fluoroquinolones and nalidixic acid. Molecular analysis indicated the presence of different small qnrD plasmids. The genetic background of the plasmids was determined and analyzed by bioinformatics. Furthermore, the plasmid stability and the resistance behavior of the plasmids were compared in E. coli, S. Typhimurium and Y. enterocolitica. Plasmid profiling revealed the presence of a previously described 2.7 kb plasmid in three and a novel 1.9 kb plasmid in one of the investigated isolates. Sequence analysis showed, that the smaller plasmid encodes a qnrD2 gene variant. In contrast to the predominant qnrD plasmid prototype, the replication stability seems to be limited over long-time periods under nonselective conditions among various genera of the Enterobacteriaceae. This study disclosed the existence of a new qnrD2 M. morganii plasmid, which is, to our knowledge, the smallest described so far.

# A06

## Spread and diversity of ESBL/pAmpC-producing *E. coli* on a German fattening pig farm

Hannah Demeke, Bernd-Alois Tenhagen, Silvia Schmoger, Annemarie Käsbohrer and Alexandra Irrgang

Bundesinstitut für Risikobewertung

*Keywords: Antimicrobial resistance, transmission, CTX-M-1, CTX-M-9, ACC-1*

Extended-spectrum β-lactamases (ESBL-) and AmpC-producing Enterobacteriaceae are widely spread in livestock and humans. The aim of this study was to analyse the diversity of ESBL/AmpC producing E. coli within a farm housing fattening pigs in six different barns. We analysed 33 samples retrospectively, as the farm was investigated originally because of the detection of carbapenemase-producing E. coli in the German AMR monitoring. We could find three different types of ESBL/AmpC-producing E. coli. A CTX-M-9 producing clone was identified in three samples of three different barns. CTX-M-1-producing isolates were identified in 11 samples from four different barns. The $bla_{CTX\text{-}M\text{-}1}$ gene was harbored mainly on ~100 kb IncI1 plasmids. XbaI PFGE cluster analysis showed no clonal relationship between these isolates. In one sample a diversity of seven different clones was determined. Only three isolates harbored the $bla_{CTX\text{-}M\text{-}1}$ gene on 32 kb IncN plasmids and were clonally related. ACC-1-producing E. coli were found in 23 samples from five barns. These isolates were closely related to VIM-1-positive strains found on the farm, but sequence analysis revealed a deletion of the $bla_{VIM\text{-}1}$ integron on the IncHI2 plasmid.
In conclusion, there was a high diversity of ESBL/AmpC-producing E. coli spread widely within the farm. Current results show that dissemination might occur through clonal spread as well as horizontal gene transfer depending on the resistance mechanism.

# A07

## Coagulase negative staphylococci as potential resistance gene reservoir for methicillin-resistant *Staphylococcus aureus* on German dairy farms

Tobias Lienen, Arne Schnitt, Heidi Wichmann-Schauer, Bernd-Alois Tenhagen

Bundesinstitut für Risikobewertung

*Keywords: MRSA, coagulase negative staphylococci, resistance gene transmission, dairy farms*

Methicillin-resistant Staphylococcus aureus (MRSA) as well as coagulase negative staphylococci (MR-CoNS) are frequently found on dairy farms. Infections caused by these bacteria cannot be treated with beta-lactam antibiotics. The objective of the study is to analyze the occurrence and genotypical characteristics of MRSA and MR-CoNS on preselected German dairy farms.
Analyses of more than 1500 samples from ten dairy farms showed that MR-CoNS and MRSA are particularly frequent in nasal swabs from calves, but less frequent in swabs from heifers or quarter milk samples from the same herds. Detection of MRSA in the quarter milk samples always corresponded to an occurrence in the bulk tank milk. According to spa typing, the detected MRSA strains were attributed to the livestock-associated CC398. Methicillin-resistance was associated with the mecA gene in SCCmec types I, IVa, IVd and V. In most cases, SCCmec types of MRSA and MR-CoNS did not correspond. However, methicillin-resistant S. aureus and S. haemolyticus isolates on three farms carried an identical SCCmec type, indicating a potential interspecies transmission of resistance genes. In contrast, all phenotypically resistant S. cohnii isolates were mecA- and mecC-negative, indicating a different genetic background of resistance.
In conclusion, monitoring should also pay close attention to MR-CoNS, since these strains frequently occur on dairy farms and might be a currently underestimated reservoir for resistance genes.

# A08

## Comparison of antimicrobial resistance patterns in *E. coli* from conventional and organic turkeys

M. Grobbel, J.A. Hammerl, A. Irrgang, A.Weiser, A.Käsbohrer* and B.-A. Tenhagen*

German Federal Institute for Risk Assessment, Berlin, Germany
*both authors contributed equally

*Keywords: antimicrobial resistance, turkey, organic husbandry, E. coli*

The common opinion is that bacteria from animals from organic farms and products thereof show fewer resistances than those from conventional husbandry. To collect representative data, turkeys from conventional (C) and organic (O) farming and their meat were investigated in the national monitoring for antimicrobial resistance in livestock and food in 2018.
Indicator E. coli were isolated from faecal samples and meat at the federal state laboratories and sent to the German Federal Institute for Risk Assessment (BfR). Minimal inhibitory concentrations (MIC) of 14 antimicrobial substances were determined following legal requirements (CID 2013/652/EU). In total 134 and 196 E. coli isolates from O and C turkey meat, and 30 and 200 from faecal samples collected on O and C farms were tested respectively.
Overall, the obtained results confirmed expectations. Isolates from O production were more frequently found to be fully susceptibility (~50% and ~20% of the E. coli from both matrices in O and C production). Similarly, for seven substances the percentage of resistant E. coli was significantly higher in C than in O production, while significantly more resistant isolates in organic than in C farming was not found. None tested isolate showed resistance to meropenem or tigecycline.
The results to some extend support the common opinion and the possible impact of husbandry conditions on resistance patterns. Differences observed also hint towards the influence of different transmission pathways.

# A09

## Four-week high zinc oxide diets: effects on commensal intestinal *Escherichia coli* populations of weaned piglets

V.C. Johanns[1], F. Ghazisaeedi[2], L. Epping[3], T. Semmler[3], B. Walther[1], L.H. Wieler[4]

[1]ZBS4 Advanced Light and Electron Microscopy, Robert Koch Institute (RKI), Berlin, Germany
[2]Institute of Microbiology and Epizootics, Free University Berlin, Germany
[3]Microbial Genomics (NG1), RKI, Berlin, Germany
[4]RKI, Berlin, Germany

*Keywords: Antimicrobial resistance, Escherichia coli (E. coli), zinc tolerance, piglets*

Currently, different strategies are used to reduce post-weaning diarrhea (PWD) associated with E. coli infections such as high-level dietary zinc oxide supplementation. However, effects of high-zinc diets on porcine intestinal bacterial populations are not fully understood yet. The aim of this work is to characterize a representative selection of E. coli isolates obtained from a high-zinc fed piglet group [HZG] and a regular fed control group [CG].
In total, 179 isolates (HZG: n=99; CG: n=80) were screened for antimicrobial resistance and zinc tolerance by determination of minimum inhibitory concentration (MIC). In addition, in silico whole genome screening (WGS) for antimicrobial resistance-, virulence- and heavy metal tolerance genes was performed using an in-house developed BLAST based screening tool (hits based on ≥ 90% identity).
Overall, porcine E. coli yielded three different levels of $ZnCl_2$ tolerance: 128 µg/ml (HZG, 2%; CG, 6%), 256 µg/ml (HZG, 64%; CG, 91%) and 512 µg/ml (HZG, 34%; CG, 3%; $p < 0.001$). More of HZG-isolates showed a resistance to Sulfamethoxazole-Trimethoprim ($p = 0.003$) compared to CG-isolates. WGS data screening for genes involved in zinc homeostasis revealed an almost ubiquitous presence of most factors tested, including zitB, zntA and pit formerly described as important for increased zinc tolerance.
Further research on mechanisms involved in development of zinc tolerance in E. coli is warranted, especially zinc-induced stress response by comparative transcriptomics.

# A10

## A kaleidoscope of phenotypes: mecC-positive Methicillin-resistant *Staphylococcus aureus* (MRSA)

Charlotte Huber[1], Lennard Epping[2], Ivonne Stamm[3], Wilma Ziebuhr[4], Gabriella Marincola[4], Markus Bischoff[5], Birgit Strommenger[6], Christiane Cuny[6], Wolfgang Witte[6], Jörg Döllinger[7], Christoph Schaudinn[1], Torsten Semmler[2], Antina Lübke-Becker[8] and Birgit Walther[1]

[1]Advanced Light and Electron Microscopy (ZBS4), Robert Koch Institute, Berlin
[2]Microbial Genomics (NG1), Robert Koch Institute, Berlin
[3]IDEXX GmbH, Ludwigsburg
[4]Institute of Molecular Infection Biology, University of Würzburg, Würzburg
[5]Institute of Medical Microbiology and Hygiene, Saarland University, Homburg
[6]National Reference Centre for Staphylococci and Enterococci, Robert Koch Institute, Wernigerode
[7]Proteomics and Spectroscopy (ZBS6), Robert Koch Institute, Berlin
[8]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin

*Keywords: mecC-MRSA, niche adaption, genome analysis*

MRSA, harboring the resistance-mediating variant mecC have strong adaptive capabilities, which enable them to colonize humans as well as a wide range of wild- and domestic animals and cause opportunistic infections.
Here we report on 45 mecC-MRSA, isolated from clinical samples of human and animal origin in Europe. Phenotypic characterization including hemolysis assays, colony spreading, toxin and biofilm production abilities as well as proteomics were performed. Whole genome sequences were investigated using MLST v-1.6, ResFinder v-2.1, VirulenceFinder v-1.6 and Geneious 11.1.5.
mecC-MRSA of animal origin were associated with six different host species, with most isolates being obtained from feline samples (73%). All isolates yielded Oxacillin minimum inhibitory concentrations between 0.5 and <4 µg/ml. Phylogenetic analysis revealed closely related genomes harboring various mobile genetic elements carrying beyond others different enterotoxins, the toxic-shock-syndrome toxin as well as further virulence factors, clustering within four clonal complexes (CC): CC599, CC130, CC1943 and CC49. However, strains of each CC yielded unexpected phenotype variations including hemolysis, colony spreading, toxin production and biofilm formation, reflecting putative differences in global regulatory systems.
Further research addressing the impact of phenotypic variation for the adaptive capability of the distinct mecC-MRSA lineages supporting their ubiquitous widespread occurrence is warranted.

# A11

## Outbreak of mastitis caused by LA-MRSA CC398 in a Bavarian dairy herd

Arne Schnitt, Tobias Lienen, Heidi Wichmann-Schauer, Bernd-Alois Tenhagen

German Federal Institute for Risk Assessment

*Keywords: methicillin, staphylococcus, MRSA, dairy, milk*

In dairy cows Staphylococcus aureus is a major mastitis pathogen. Methicillin resistant Staphylococcus aureus (MRSA) have been rarely detected on dairy farms around the world. In a field study, quarter milk samples (QMS) from dairy cows, swab samples from young stock and environmental samples were collected from German dairy farms and screened for the presence of methicillin resistant staphylococci. On a smallholder dairy farm in Bavaria, a relatively high MRSA prevalence of 14.8% (4/27) was detected within the herd. The average herd somatic cell count (SCC) in QMS was 115,000 cells/ml and quarters that carried MRSA had an average SCC of 531,000 cells/ml. MRSA were also detected in the bulk tank milk and in nasal swabs from calves, heifers and pigs. In the environment, MRSA were detected in samples from the milking equipment. Milkers were not using gloves, one udder towel was used for all cows and no milking-cluster disinfection was performed. All MRSA were classified as clonal complex 398 and spa-types t011 and t1451.
The same MRSA strain causes mastitis in cows and seems to spread among different groups of animals. However, MRSA spa-types from pigs (t1451) and cattle (t011) were different. Improper milking hygiene procedures were observed, indicating a potential risk factor for MRSA transmission during the milking process. The MRSA detection in the bulk tank milk might be of human health concern since the farmer's family and some neighbors consume raw milk from that herd.

# A12

## Antimicrobial usage in horses: Developing and establishing a method for evaluating electronical data and first results

Anne Schnepf, Astrid Bienert-Zeit, Karsten Feige, Lothar Kreienbrock

University of Veterinary Medicine Hannover

*Keywords: horses, Germany, antimicrobial usage, electronic practice management software*

As resistant bacteria are transmissible between animals and humans, analysis of data on antimicrobial usage (AMU) is vital. While several AMU monitoring programs in veterinary medicine exist for farm animals in Germany, there is no system for horses.
The aim of this study is to show the benefit and possibilities of evaluating AMU data generated by the electronic practice management software (EPMS). Hence, the number of antibiotics, amount and percentage of active ingredients used were calculated in the Clinic for Horses, TiHo, within a feasibility study.
In 2017, 6,489 antimicrobial drugs were administered to 837 horses. In total, 161.27 kg of active ingredients were documented. Antibiotic classes used most often were sulphonamides (84.32 kg; 51.95 %), penicillins (30.11 kg; 18.55 %) and nitroimidazoles (24.84 kg; 15.3 %). Regarding critically important antibiotics (CIA), the proportion of used CIA with highest priority was 0.14 % (0.22 kg), and 20.85 % (33.85 kg) of CIA with high priority.
Results show that data generated by EPMS can be evaluated with comparatively little effort. For a realistic analysis, close cooperation with the respective veterinarians is essential.
The method developed can also be applied to evaluate data from EPMS of other clinics or animal species like pets. With this, a more detailed discussion on AMU in companion animals is possible.

# A13

## Development of a multiplex PCR assay using orphan genes as markers for the identification of individual *Escherichia coli* strains after mixed inoculations

M. Ferrandis Vila[1], C. Berens[1], S. K. Tiwari[2], B. Van der Putten[3,4], N. V. Trung[5], M. Bootsma[7], R. M. La Ragione[8], S. Matamoros[4], N. T. Hoa[5,6], J. Alvarez[9,10], D. Jolivet[3], J. Ritchie[8], A. Fruth[11], J. Leng[8], S. Schwarz[12], L. Domínguez Rodríguez[9,10], M. Ugarte-Ruiz[9], A. Bethe[12]; A. Fivian-Hughes[8], T. Semmler[2], C. Schultsz[3,4], C. Menge[1]

[1]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena (Germany);
[2]Robert Koch Institute, Microbial Genomics, Berlin (Germany);
[3]Amsterdam UMC, Department of Global Health, Amsterdam (the Netherlands);
[4]Amsterdam UMC, Department of Medical Microbiology, Amsterdam (the Netherlands);
[5]Oxford University Clinical Research Unit (Vietnam);
[6]Nuffield Department of Medicine, University of Oxford, Oxford OX3 7BN (United Kingdom);
[7]Mathematical Institute and UMCU, Utrecht University, Utrecht (the Netherlands);
[8]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, Guildford (United Kingdom)
[9]VISAVET, Health Surveillance Center, Universidad Complutense de Madrid, Madrid (Spain);
[10]Animal Health Department, Veterinary Faculty, Complutense University of Madrid, (Spain);
[11]Robert Koch Institute, Enteropathogenic Bacteria and Legionella, Wernigerode, (Germany);
[12]Institute for Microbiology and Epizootics, Freie Universität Berlin, Berlin (Germany);

*Keywords: E. coli; Escherichia coli; Antimicrobial resistance; Host restriction*

The prevalence of antimicrobial resistance (AMR) has increased alarmingly. Commensal bacteria in healthy human and animal populations are an important reservoir of AMR genes. AMR transmission within E. coli is dominated by certain lineages. To what extent these are restricted to certain host species and how host restriction impacts on the degree of transmission of resistant E. coli between hosts remains unknown. This study aims to unveil the genetic patterns of E. coli strains relevant for host restriction and transfer efficiency of plasmid-borne AMR genes by highly-parallel testing in biologically relevant models.
To qualitatively and quantitatively assess the fate and genetic stability of single strains after cocktail inoculation, discriminatory orphan genes (ORFans) were chosen as strain-specific markers. From a sequenced set of 1199 E. coli collected in European countries and Vietnam, 28 strains in possession of ESBL genes on AMR plasmids but of few or no known virulence factors, were selected as a sample of isolates specifically adapted to chicken, cattle, pig and human hosts and. Five ESBL strains with no identifiable host preference were selected as generalist strains. Twenty of the strains possessed ORFans used for primer design and generation of a series of PCR multiplexes specific for bacteria of each host type. Validation studies with spiked fecal matter from pigs showed that this approach allowed successful identification of all strains in a complex matrix.

# A14

## Characterization of bla$_{CTX-M-1}$ gene regions on plasmids from *Escherichia coli* isolates collected in the GERM-Vet resistance monitoring program 2008-2015 from diseased food-producing animals

A.-K. Schink[1], G. B. Michael[1], D. Hanke, H. Kaspar[2], L. Falgenhauer[3], C. Imirzalioglu[3], T. Chakraborty[3], S. Schwarz[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Germany
[2]Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany
[3]Institute of Medical Microbiology, Justus-Liebig-University Gießen and German Center for Infection Research (DZIF), Partner site Giessen-Marburg-Langen, Gießen, Germany

*Keywords: bla$_{CTX-M-1}$ gene regions, Escherichia coli, food-producing animals*

**Background and objectives:** The aim of the study was to analyse the genetic environment of plasmid-borne bla$_{CTX-M-1}$ genes of Escherichia coli isolates from diseased food-producing animals.
Materials and methods: The ESBL gene bla$_{CTX-M-1}$ was identified in 352 of 7,810 E. coli isolates from diseased food-producing animals collected in GERM-Vet (2008-2015). Fifty representative isolates were subjected to plasmid transfer experiments and whole genome sequencing. The transformed plasmids were characterized by PCR-based replicon typing and PCR assays for the detection of co-located resistance genes.

**Results:** The plasmids were positive for nine different replicons or replicon combinations, one plasmid was non-typeable. ISEcp1, bracketed by 5-bp direct repeats, was associated with bla$_{CTX-M-1}$ on most IncI1 plasmids. On IncN, IncFII and most IncF plasmids ISEcp1 was truncated by IS26 in the upstream region and downstream a Δmrx-mph(A) cluster was detected followed by IS26 at varying positions in the sequences of different plasmids. Commonly detected co-located antimicrobial resistance genes conferred resistance to sulphonamides, aminoglycosides and tetracycline.
Conclusions: The genetic environment of the bla$_{CTX-M-1}$ genes was diverse, but similarities among the different plasmid families were noted. IncN, IncI1 and IncF plasmids play a role in the dissemination of bla$_{CTX-M-1}$; the co-located antimicrobial resistance genes may facilitate the spread and persistence of this ESBL gene.

# A15

## P90RSKactivation during the influenza virus infection triggers the nuclear export of the viral genome

André Schreiber, Darisuren Anhlan, Tim Krischuns, Laurita Boff, Stephan Ludwig

*Keywords: Influenza, nuclear export, Raf/MEK/ERK pathway, p90RSK, nucleoprotein*

The Raf/MEK/ERK/RSK signaling cascade is activated during the influenza virus infection to support th e nuclear export of newly synthesized viral ribonucleoproteins (vRNP). The aim of this study is to reveal the exact mechanism of how this kinase pathway contributes to the vRNP export
Therefore, the pathway was manipulated with specific inhibitors (MEK: CI-1040, RSK: BI-D1870) and siRNAs (siERK, siRSK). Nuclear distribution of viral proteins was analyzed by chromatin fractionation and super-resolution microscopy (STORM). Mass spectrometry technique and generation of viral mutants where used to analyze the importance specific serine residues within the viral nucleoprotein (NP)
In the presence of the kinase inhibitors nuclear vRNPs get stuck at the chromatin, caused by a reduced interaction of the vRNPs with the viral matrix protein M1. Missing NP phosphorylation of two serine residues (S269, S392) might explain the decreased binding with M1. RSK inhibition had a broad anti-viral effect by reduction of viral titers and nuclear retention of vRNPs for all tested IV, including 2009 pandemic swine flu, highly pathogenic bird flu (H5N1, H7N9) and influenza B. This indicates the importance of the viral pathway activation for the assembly of the vRNP export complex at the chromatin by RSK-dependent NP phosphorylation and providing of an interaction site for the M1 protein.

# A16

## More than just a weed killer: exposure to Roundup selects for resistance with low fitness costs in pathogenic enteric bacteria *Escherichia coli* and *Salmonella enterica* from farm animals

K. Bote[1], J. Poeppe[1], Paul Johnston[2,3,4], U. Roesler[1], O. Makarova[1]

[1]Institute of Animal Hygiene and Environmental Health, Centre for Infection Medicine, Freie Universität Berlin, Berlin, Germany
[2]Evolutionary Biology, Institute for Biology, Freie Universität Berlin, Berlin, Germany
[3]Berlin Center for Genomics in Biodiversity Research, Berlin, Germany
[4]Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany

*Keywords: Roundup, glyphosate, experimental evolution, resistance, fitness costs*

Background and objectives: The herbicide glyphosate, the active ingredient in the formulation Roundup (RU), is currently the most-used herbicide in the world. Additionally, it is patented as an antimicrobial and its effects on microbes are currently investigated. Both Escherichia coli (E. coli) and Salmonella enterica are important zoonotic Enterobacteriacea which can be exposed to glyphosate residues via feed. Therefore, we investigated whether RU can induce bacterial resistance and if it impacts their fitness.
Materials and methods: Ten isolates each of E. coli and Salmonella enterica serovars from pigs and cattle were passaged daily at increasing concentrations of RU. Stable resistant isolates and respective ancestors were whole genome sequenced and growth dynamics analysed.

Results: The overall dynamics of adaptation to RU was slow and relatively low-level, with early extinctions in E. coli. One E. coli and four Salmonella isolates showed a 2-4-fold increase in minimum inhibitory concentration to RU. Mutations associated with glyphosate resistance were found in all Salmonella isolates but not in E. coli. Mostly no fitness costs were found.

Conclusion: Salmonella are more likely to develop resistance to RU compared to E. coli. Although RU resistance does not occur easily and is relatively low, resistant mutants show no fitness costs. This suggests that RU may result in preferential selection of pathogenic Salmonella bacteria that can persist in the environment.

# A17

## Characterization of recently emerging "high risk" *Klebsiella pneumoniae* ST307 isolated from the urinary tract of a dog

A. Bethe[1], I. Eichhorn[1], C. Weingart[2], N. Roschanski[3], A.-K. Schink[1], J. Brombach[1], B. Walther[4], B. Kohn[2], A. Lübke-Becker[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany
[2]Small Animal Clinic, Freie Universität Berlin, Berlin, Germany
[3]Landeslabor Schleswig-Holstein, Neumünster, Germany
[4]Robert Koch-Institute, Berlin, Germany

*Keywords: K. pneumoniae, ST307, multidrug resistant, canine UTI, chronic infection*

Klebsiella pneumoniae (Kp)-infections in humans and animals are frequently associated with multi-drug resistant (MDR) phenotypes. In human medicine, the global spread of MDR Kp is linked to successful high-risk genetic lineages such as ST258 and the newly emerging ST307. Here, we report on multiple isolations of ST307 from a dog suffering from chronic urinary tract infection (UTI).
All confirmed Kp isolates were whole-genome sequenced (WGS) using Illumina MiSeq. Genotypic characterization included the determination of sequence type (ST), transferable resistance genes and plasmids (https://cge.dtu.dk).
Ten Kp isolates were identified, with five being in mixed culture with other bacterial species. All Kp belonged to ST307, six harbored an IncF-plasmid and were ESBL-positive ($bla_{CTX\text{-}M\text{-}15}$). First phylogenetic analysis revealed a close relationship between the strains.
Although genetic features that may provide an advantage in adaption to the human host have been reported for this lineage, $bla_{CTX\text{-}M\text{-}15}$-MDR ST307 can cause UTI in companion animals. This is in accordance with a current study reporting ST307-$bla_{CTX\text{-}M\text{-}15}$ in canine and feline urine samples. As they can serve as source of infection for high-risk Kp clones, companion animals may pose a risk to their owners. Consequently, further investigation is needed to elucidate whether bacterial persistence may explain the detection of eight closely related isolates from a canine patient under antibiotic therapy in a one-year period.

# A18

## Evidences of overlapping between antimicrobial resistance and drug usage surveillance and monitoring systems in the Human, Animal and Food Sectors in European countries

Octavio Mesa-Varona, Bernd-Alois Tenhagen

German Federal Institute for Risk Assessment, Berlin, Germany

*Keywords: Antimicrobial use, Antimicrobial resistance, human, animal, food*

**Background**: Antimicrobial resistance (AMR) and use (AMU) in the human and animal sectors together with the resistance patterns found in food are major public health concerns. Surveillance and monitoring systems are essential to control and assess the trends.
**Methods:** Relevant data sources such as peer-reviewed articles, databases, national and European grey reports among others were identified by a thorough review in order to identify public information about monitoring and surveillance systems and their databases on AMU and AMR in humans, animals and food.
**Results:** Several overlapping between systems on AMR and AMU have been found in the human, livestock and food sectors. In addition, disparities between countries and sectors in the AMU section has been encountered in relation to data sources and units. Moreover, diverse sample types, units, standards, evaluation criteria and laboratory methods have been detected in the AMR section.

**Conclusions:** The lack of standardization among the human, animal and food sectors and across countries may contribute to hamper the One Health worldwide strategy. Current overlapping between monitoring and surveillance systems may provide room for streamlining and harmonization.

# A19

## Antibiotic usage pattern in broiler chicken flocks in Germany

Svetlana Kasabova, Maria Hartmann, Ertugrul Hatice, Katharina Hommerich, Käsbohrer Annemarie, Lother Kreienbrock

Institut für Biometrie, Epidemiologie und Informationsverarbeitung, Stiftung Tierärztliche Hochschule Hannover, Germany

*Keywords: antimicrobial usage, broiler chicken flock, treatment frequency, treatment incidence, used daily dose*

Within the longitudinal VetCAb-Sentinel (Veterinary Consumption of Antibiotics) project antimicrobial usage data at farm level from 2436 commercial broiler chicken flocks between 2013 and 2018 were analyzed, aiming to investigate the real weight of the broiler chickens at the time of treatment, the treatment frequency (TF) as well as the proportion of the different antimicrobial classes of the overall TF.
The median age of the broiler chicken flocks at the time of treatment was 5 days, which correspond to a median weight at the time of treatment of 111g with significant differences between antimicrobial classes. The median TF was 6 and veterinary medical products belonging to nine different antimicrobial classes were used. Over the six years a significant increase in the usage of lincosamides and aminoglycosides could be observed, while the percentage of the TF for fluoroquinolones, macrolides and polypeptides is on the decrease.
Our results show that the median weight of the broiler chickens at the time of treatment is 889g lower than the standard weight for broilers proposed by ESVAC. It may vary considerably and can have an impact on the standardization procedure. Additionally, decreasing usage of highest priority critically important antimicrobials, such as fluorquinolones, macrolides and polypeptides in broiler chicken flocks could be shown, probably as a consequence of an increasing awareness of the global antibiotic resistance situation.

# A20

## The occurrence of LA-MRSA and ESBL in different swine farming systems - a subproject of the #1Health-PREVENT project

S. Linnemann, H. Müller, I. Kobusch, M. Boelhauve

South Westphalia University of Applied Sciences, Germany

*Keywords: LA-MRSA, ESBL*

Background and objectives: During past years multidrug-resistant microorganisms (MDRO) in livestock become the focus of attention. The increasing occurrence of livestock-associated (LA) LA-MRSA and extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL) in swine farms leads to an overview study of the prevalence depending on different husbandry systems.
Materials and methods: This research was performed on swine farms with conventional husbandry, alternative systems and husbandries with ecological guidelines. Environment and fattening pigs were analyzed at two different time points.
Results: Conventional systems showed a highly increased prevalence for LA-MRSA in both environment and pigs. Interestingly, pigs in farms with alternative and ecological husbandry revealed a significant absence of LA-MRSA findings at the end of the fattening. ESBL was verified in all systems with an elevated occurrence in conventional farms.
Conclusion: Results suggest that alternative and ecological housing systems promote a lower rate of LA-MRSA and ESBL. Potential influences like straw bedding material, execution of cleaning and disinfection as well as open airing could possibly create a more complex microbiota in the environment with more bacterial competition against MDRO. The #1Health-PREVENT project investigates these influences for a better understanding of the above mentioned interactions to define an effective strategy to reduce MRDO occurrence in livestock-breeding including optimal and practical housing conditions.

# A21

## Efficacy testing of six disinfection methods for hatching eggs against ESBL producing *E. coli* using eggshell samples as a carrier model

Gerzon Motola, Sarah Brüggemann-Schwarze, Hafez M. Hafez

Institute of Poultry Diseases - Freie Universität Berlin

*Keywords:Eggs, Disinfection methods, ESBL, E. coli*

Introduction: The presence of ESBL (extended-spectrum beta-lactamase) producing bacteria on poultry products is an important subject for veterinary and human health due to the zoonotic infection risk for producers and consumers. The present study focuses on testing the efficacy of 6 different disinfection methods on hatching eggs, aiming to reduce ESBL producing contaminants at the very beginning of the production pyramid.
Materials and Methods: Sterile eggshells cutouts were artificially contaminated with $10^8$ ESBL producing E. coli (CTX-M-1, isolate from 1-day-old chicks) and used as carrier models for each disinfection method. The contaminated samples were separated in 2 groups; disinfected and non-disinfected. Disinfections were performed following the products specifications. Each eggshell sample was separately crushed and bacteria re-isolated. Re-isolation rates were compared and the disinfection efficacy determined.
Results: The tested methods: 1) Formalin gassing, 2) hydrogen peroxide-alcohol spray, 3a) essential oils spray, 4) peracetic acid foam and 5) low energetic electron radiation were able to reduce (at least 2logs) or eliminate the initial ESBL contamination. These results demonstrate the disinfection efficacy against ESBL producing E. coli, decreasing the risk of multi-resistant bacteria in one-day-old broiler chicks. 3b) Essential oils as cold fog did not reach the efficacy threshold. The five efficient methods were chosen for subsequent animal trials.

# A22

## Effects of straw bedding on conventional LA-MRSA positive pigs during fattening period

H. Müller[1], I. Kobusch[1], S. Linnemann[1], N. Effelsberg[2], A. Mellmann[2], M. Boelhauve[1]

[1]South Westphalia University of Applied Sciences, Germany
[2]Institute of Hygiene, University of Münster, Germany

*Keywords: LA-MRSA, decolonization*

Background and objectives: Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) is widely spread among livestock. Strategies against this increasing development are needed.
Materials and methods: This research was performed on a conventional swine farm for fattening pigs with open airing and straw bedding. Two stables were prepared as follows: simple cleaning (SC) and cleaning with disinfection (CD). Nasal LA-MRSA colonization of the pigs was analyzed directly after arrival on the farm and subsequently during the fattening period as well as environmental contamination of LA-MRSA. Additionally, nasal swab of selected animals for metagenome analysis were taken.
Results: At the beginning, all pigs were tested positive for LA-MRSA. Further screenings showed comparable results in the stables. Both, pigs and environment were frequently tested negative for LA-MRSA after a few weeks. Interestingly, group SC showed a decrease of LA-MRSA-negative findings of pigs after 11 weeks with a total conversion to LA-MRSA negativity at the end of the fattening period after 14 weeks like in group CD.
Conclusion: It seems that the process of decolonization is not mainly influenced by the preparation of the stables. The straw bedding with a diverse microbiome could be an important factor influencing the nasal colonization with LA-MRSA and the elimination at the end of the fattening. Metagenome analysis (data in progress) of nasal swabs and straw samples will likely give a hint for this interaction for a better understanding and the development of an effective strategy against the spread of LA-MRSA.

# A23

## Cyclophilin blockers inhibit coronavirus replication and regulate expression of host defense-related genes in primary human bronchial epithelial cells

Y. Ma-Lauer[1,2], Z. Yu[1,2], K. Pusl[1,2], S. Schwinghammer[1,2], B. von Brunn[1,2], M.-G. Stoleriu[3], A. Schamberger[3], M. Nakayama[3], C. Staab-Weijnitz[3], A. von Brunn[1,2]

[1]Max von Pettenkofer-Institut, LMU München
[2]DZIF, Standort München
[3]Comprehensive Pneumology Center (CPC), Helmholtz Zentrum München

*Keywords: cyclophilin inhibitors, corona virus infection, primary human bronchial epithelial cells, cellular genes as antiviral targets*

We have demonstrated earlier that cyclophilin inhibitors abolish coronavirus (CoV) replication in susceptible cancer cell lines. Primary human bronchial epithelial cells (pHBECs) represent an ideal model for CoV infection close to the in vivo situation in humans. pHBECs isolated from three patients, were infected with HCoV-229E and HCoV-NL63 and treated with immunosuppressive cyclosporin A (CsA) and non-immunosuppressive Alisporivir (ALV) cyclophilin inhibitors. Our results show that CsA as well as ALV dramatically inhibit viral replication without impairing viability of the primary cells. This indicates that ALV is a very promising drug candidate without immunosuppressive side effect to treat HCoVs-infected patients. In addition, antiviral factor p53 antagonizing HCoV replication is clearly upregulated upon treatment of infected cells with CsA and ALV at the protein level, suggesting mechanisms of drug action on viral replication. In contrast, the protein level of cyclophilin B strongly decreases after treatment of CsA and ALV. A number of immunoresponsive genes were also tested at the mRNA level after infection and drug treatment and will be discussed.

# A24

## Nasal MRSA colonization of veterinary staff in German equine clinics

Christiane Cuny[1], Wolfgang Witte[1], Ute Pansegrau[2], Wolfgang Scheidemann[3]

[1] Robert Koch Institute, Section Nosocomial Infections and Antibiotic Resistance, 38855 Wernigerode
[2] Animal Hospital Lüsche, 49456 Bakum
[3] Veterinary Competence Centre Karthaus, 48249 Dülmen

*Keywords: MRSA, horse clinics, colonization of staff*

Nosocomial infections in equine clinics are common in many countries and associated with particular „hospital strains". When emerging in equine clinics these MRSA are also found as nasal colonizers of veterinary staff. Nasal MRSA carriage bears the risk for infections in case of predisposing conditions (e.g. wounds). Here we report a study on nasal MRSA colonization of 325 staff members in 16 German equine clinics, on the frequencies at which barrier precautions were implemented, on conditions predisposing to MRSA carriage, and on results from typing the isolates.
The average prevalence of MRSA colonization was 17.5%. Wearing gloves when carrying out relevant activities was reported by 44% of the 143 veterinarians and by 33% of the other 182 staff members. Among the veterinary assistants MRSA carriage was less frequent when wearing gloves (8.3% vs. 22%, p=0.044). Face masks were used by 8% of the veterinarians and 12.5% of veterinary assistants. Antibiotic consumption in the time period of 6 month prior to sampling proved to be a risk factor (p=0.001) for MRSA colonization.
Among the 125 MRSA isolates 91% represented the veterinary hospital associated subpopulation of clonal complex CC398.
The prevalence of nasal MRSA colonization of veterinary staff is still high and above that observed for staff in other veterinary disciplines besides that attending conventional livestock. Results from typing indicate that acquisition seems to be mainly associated with work in the clinics.

# A25

## Comparison of antimicrobial resistance of thermophilic Campylobacter isolates from conventional and organic turkey meat

Tenhagen, B.-A. [1], Alt, K. [1], Thieck, M. [1], Käsbohrer, A. [1,3], Kollas, C.[1], Pfefferkorn, B.[2], Naumann, S. [2], Stingl, K. [1]

[1] Bundesinstitut für Risikobewertung, Abteilung Biologische Sicherheit, Berlin
[2] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin
[3] Veterinärmedizinische Universität Wien, Institut für Veterinary Public Health, Wien

This study tested the following hypothesis: Antimicrobial resistance (AMR) is lower in Campylobacter isolates from organic than from conventional turkey meat.
Samples of turkey meat at retail were collected in retail shops all over Germany proportionate to the human population in the respective land. Samples were tested in the regional laboratories of the Länder according to ISO 10272-1:2017.
Identified Campylobacter spp. were confirmed at the National Reference Laboratory for Campylobacter and isolates were tested for AMR according to the prescriptions given in Commission Implementing Decision (CID) 2013/652/EU. Prevalence of Campylobacter spp. in the samples was calculated as the proportion of positive samples among the tested samples. The comparison of AMR between the two bacterial species and sample types was carried out using logistic regression analyses using resistance to a specific antimicrobial as the binary outcome and the bacterial species and the source of the meat as fixed factors. The sampling location, "Land", proved to be a non-significant factor in all analyses and was, therefore, removed from the models.
Prevalence of Campylobacter spp. was higher in organic (80/245, 32.7 %) than in conventional meat (102/527, 19.4 %). The proportion of fully susceptible isolates in turn was higher in isolates from organic (38.9 %, 28/72) than from conventional meat (17.4 %, 16/92). Results confirm a higher resistance in Campylobacter spp. isolated from conventional as compared to organic turkey meat.

# A26

## Developing a global dynamic dashboard for antimicrobial resistance related research and development

Ralf Sudbrak, Jennie Hood, Magda Moutaftsi, Suzanne Edwards, Elmar Nimmesgern

Scientific Programme Officer Global AMR R&D Hub c/o Unicorn Workspaces

*Keywords: antimicrobial resistance; antibiotics; research investment*

The Global AMR R&D Hub was launched in May 2018, following a call from the G20, to address challenges and improve coordination/collaboration in global AMR R&D. It is currently a partnership of 15 countries, the EC and two philanthropic foundations who form the Board of Members. The Global AMR R&D Hub will support global priority setting and evidence-based decision-making on allocation of resources for AMR R&D. A strong global evidence base that captures AMR R&D happening in all One Health sectors is required to inform this priority setting. To address this knowledge gap, the Global AMR R&D Hub is developing a dynamic dashboard that will present information on AMR R&D.
The methodology for the development of the dashboard will be presented including how:

- existing initiatives collecting AMR R&D project information were identified, built on and incorporated
- information of AMR R&D projects was systematically collected
- consistency of categorizations was ensured and applicable across all One Health sectors, and
- the information will be presented in an interactive and user-friendly way.

The dashboard will collect close to real time AMR R&D information from human, animal, plant and environment sectors. This will include public and private funded basic and applied R&D on therapeutics, preventives and diagnostics as well as operational and policy research. The first stage of the dashboard, capturing AMR R&D information on human bacterial infections will be launched in December 2019.

# A27

## DNase treatment to reduce Campylobacter - and Pseudomonas-biofilms

Christoph Püning, Thomas Alter, Greta Gölz

Institute of Food Safety and Food Hygiene, FU Berlin

*Keywords: Campylobacter, Biofilm, DNase*

Campylobacter (C.) has the ability to colonize and survive in the microaerobic milieu of existing biofilms, which might lead to cross-contamination along the food chain. The reduction of biofilms with DNases can be a potential application to reduce the risk of cross-contamination. Single species biofilms of C. jejuni and Pseudomonas aeruginosa as well as mixed biofilms of both species were grown in 96 well polystyrene plates in Mueller-Hinton broth for 72 h at 37 °C under microaerobic conditions, before several DNase I (2 U/ml) treatments were investigated: DNase I in water i) without a previous washing step, ii) after a previous washing step or iii) in DNase buffer after a previous washing step. Afterwards the biofilm mass was determined by crystal violet staining and measurement of the absorbance. The treatment with DNase I in water without a previous washing step reduced the biofilm mass of all three biofilms. Even though DNase I treatment in water with a previous washing step reduced the mono-species biofilm of C. jejuni, but neither the Pseudomonas mono-species nor the dual-species biofilm mass was reduced. However, if DNase was applied in buffer, the mass of all three biofilms were reduced, even with a previous washing step before DNase I application.Therefore, despite the knowledge about the bacterial composition of existing biofilms along the food chain, it is important to use defined conditions for the DNase I treatment to efficiently reduce the existing biofilms.

# Poster Presentations Parasitic Zoonoses

## P01 – P09

# P01

## Prevalence of *Toxoplasma gondii* and *Alaria alata* in Game Animals in Brandenburg, Germany

N. S. Bier[1], K. Stollberg[2], C. Kästner[1], K. Nöckler[1], A. Mayer-Scholl[1], A. Johne[1] and M. Richter[1]

[1]German Federal Institute for Risk Assessment (BfR), Department for Biological Safety, Berlin, Germany
[2]German Federal Institute for Risk Assessment (BfR), Department Safety in the Food Chain, Berlin, Germany

*Keywords: Toxoplasma gondii, Alaria alata, deer, wild boar*

Toxoplasma gondii and Alaria spp. are parasites with zoonotic potential that are prevalent in wildlife. To better estimate the public health risk emanating from these parasites in game, this study assessed their occurrence in game animals in the German Federal state of Brandenburg over a period of two years. During the hunting seasons 2017/2018 and 2018/2019, 22 driven hunts were organized in 15 different hunting grounds in cooperation with the Frankenförder Forschungsgesellschaft and the German Bundesforst. In the framework of these hunting events, wild boars, roe deer and red deer were sampled. For the direct detection of Alaria alata and T. gondii, samples of tongue, abdominal fat, and muscle tissue of diaphragm, foreleg, masseter muscles, and myocardium were examined. For serological analysis, blood samples were taken from the abdominal cavity. Direct detection of T. gondii was conducted using molecular methods. Mesocercariae of A. alata were detected using the Alaria spp. mesocercariae migration technique. Serological examination of roe deer, red deer and wild boar revealed T. gondii-specific antibodies in 12.8% (16/125, 95%CI: 7.5-20%), 4.3% (2/47, 95%CI: 0.5-14.5%), and 23.9% (43/180, 95%CI: 17.9-30.8%) of the samples, respectively. Mesocercariae of A. alata were found in 27% of 232 tested wild boars. As T. gondii and A. alata are autochthonous in game in the studied hunting areas, meat products should undergo proper heating prior consumption.

# P02

## Occurrence of *Cryptosporidium spp*. in game and raw consumed food, in Germany – is there a potential human health relevance?

Claudia Jäckel, Karsten Nöckler, Martin Richter, Carl Gremse, Jens-Andre Hammerl, Anne Mayer-Scholl

German Federal Institute for Risk Assessment

*Keywords: Cryptosporidium, game, salad, herbs, prevalence*

Cryptosporidiosis is a widespread human/animal diarrheal disease, caused by the protozoan Cryptosporidium. The parasite is mainly ingested by consuming contaminated water or raw food, contaminated by the contact with faeces of infected persons or animals. However, there is only insufficient information on the worldwide distribution of Cryptosporidium.
For a better understanding of the risk of human cryptosporidiosis by consumption of contaminated food in Germany, this study investigated the occurrence in game and food products with low processing degree (i.e. salads, herbs). Extracted samples were molecularly checked for the presence of three gene sequences (18S rDNA, GP60, COWP) using nested PCR and specified by sequencing and RFLP. In addition, salads and herbs were also examined by fluorescence microscopy. Altogether 229 wildlife samples from eight districts in Brandenburg (hunting season 2017/18), as well as 114 salads/herbs were tested for the presence of Cryptosporidium.
First results indicate that the prevalence of Cryptosporidium in game of Brandenburg is high (wild boar: 24.11%, red deer: 10.53%, roe deer: 42.37%), whereas young animals seem to be more affected than older. Additionally to the wild boar and deer specific species C. scrofarum, C. suis, and C. sp. deer specific genotype, human pathogenic species C. parvum and C. ubiquitum were also identified. In contrast to game, Cryptosporidium could neither be molecularly nor microscopically detected in salads/herbs.

# P03

## Effects of Sphingolipids Derivatives on *Leishmania spp*. Viability and Virulence

Sharareh Salehi Hossainy, Toni Aebischer, Christoph Arenz, Geo Semini, Essa M.Saied

Robert Koch Institute

*Keywords: Leishmania, inositol phosphoryl ceramide, phosphosphingolipid phospholipase C-like, neutral SMase, ceramide-based synthesized compounds*

**INTRODUCTION:** Leishmaniasis is a neglected tropical disease that is caused by the protozoan par asite Leishmania a unicellular parasitic genus of kinetoplastids. Around 12 million people are affected by this disease worldwide. The parasite is transmitted by the bite of infected female phlebotomine sandflies that feed for blood. Leis hmania's life cycle alternates between flagellated promastigotes living in the midgut of sandflies and non-flagellated amastigotes residing in mammalian phagocytes. Current disease treatment relies on substances characterized by significant toxicity, limited efficacy, and high cost. Understanding parasite biology and host interaction in greater detail is expec ted to result in the discovery of new drug candidates. Sphingolipids (SL) are essential components of eukaryotic cell membranes with key roles in cell processes like signal transduction, intracellular membrane trafficking and the regulation of cell growth and survival. Leishmania species synthesize inositol phosphoryl ceramide (IPC) which is derived from ceramide as their primary PSL which is distinct to the host cell SL. IPC-based SLs are found also in plants but not in mammals. However, IPC metabolizing parasite enzymes such as Inositol phosphosphingolipid phospholipase C-like (ISCL) proteins are homologous to human neutral SMase and have a role in hydrolysis of both IPC and (host) SM. Result s of reverse genetic studies (conducted by Zhang in 2010) showed that parasites deficient in ISCL synthesis became apat hogenic.

**PURPOSE:** Therefore, this study evaluates whether synthetic sphingolipid-derivatives can be used to affect Leishmaniaviability and pathogenicity and, if true, will aim at elucidating the molecular basis of this effect.

**METHODS:** Four candidate compounds (Ala-007, Ala-Diazirine, ES048, and C11AG) were synthesized and tested both, in axenic culture (against promastigotes of L.major and L.mexicana) and against amastigotes in the model of in vitro infected bone-marrow derived macrophages (BMDM) of C57BL/C mice. Besides, the cytotoxicity assay has been conducted against confluent BMDM to analyze whether ceramide-based synthesized compounds have toxic effects on host or not. Furthermore, a model of Leishmania infection of human cells was adapted to study host-parasite interaction also in human cells. We isolated monocytes from peripheral mononuclear cells (PBMCs) and quantified IFN-ɣ, IL-10, IL- 4 after 24hrs of infection to establish the base line data of this model.

**RESULTS:** All four compounds inhibit leishmania proliferation with IC50 between 0.8 and 1.9 µM. IC50 values were in the same range testing pro-and amastigotes, respectively. The results demonstrated that ceramide-based synthetic compounds did not show significant toxic effect on host in the same IC50 values. Besides, both the peripheral blood mononuclear cells (PBMCs) and monocytes infection with the stationary phase of L.major promastigotes did not result the significant cytokine responses after 24 hours post infection.**CONCLUSION:** Candidate compounds targeting the SL metabolism show in vitro reactivity against Leishmania spp..

# P04

## Molecular characterization of Giardia duodenalis isolates in Germany

Woschke Andreas[1], Faber Mirko[2], Stark Klaus[2], Regnath Thomas[3], Sobottka Ingo[4], Aebischer Toni[1], Ignatius Ralf[4,5], Klotz Christian[1]

[1]Robert Koch Institute, Department of Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany
[2]Robert Koch Institute, Department of Gastrointestinal Infections, Zoonoses and Tropical Infections, Berlin
[3] Laboratory Enders and Partners, Stuttgart
[4] LADR GmbH, Medizinisches Versorgungszentrum, Lauenburger Str. 67, 21502 Geesthacht, Germany
[5]Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Institute of Health, Institute of Microbiology and Infection Immunology, Berlin, Germany.
[6]Labor 28 GmbH, Berlin, Germany

*Keywords: Molecular characterization, Giardia duodenalis*

Background and objectives: The eukaryotic parasite Giardia duodenalis causes diarrheal disease in humans worldwide and is an important public health concern. Eight distinct genetic groups (referred to as assemblage A-H) are described, of which A and B are pathogenic to humans. This pilot project aims at applying the latest typing methods in order to discriminate assemblages and sub-assemblages of G. duodenalis from autochthonous and travel-associated infections in Germany.Materials and methods: The assemblage types were classified by a common multi locus sequence genotyping (MLST) scheme at the glutamate dehydrogenase, triose phosphate isomerase and beta-giardin gene.

Results: More than 120 G. duodenalis positive samples were successfully typed at one or more loci. Approximately 70% belonged to assemblage B, whereas assemblage A was found in approximately 30% of the cases. About 50% of the cases were travel-associated. Preliminary analysis indicated no significant differences in genotype between autochthonous and foreign-acquired G. duodenalis isolates. Further sub-classification is currently being performed.

Conclusion: At the level of assemblage type, no difference was observed between autochthonous and travel-associated giardiasis cases. The presented data lay ground for a more detailed genotype analysis with higher discriminatory power.

# P05

## Preventative measures used against L. infantum infection in a highly endemic countries: a retrospective study of 2837 dogs (2012-2018)

Marta Baxarias[1], Josep Homedes[2], Cristina Mateu[2], Laia Solano-Gallego[1]

[1]Universitat Autònoma de Barcelona
[2]Ecuphar veterinaria SLU

*Keywords: Canine leishmaniosis, prevention, repellent, vaccine, domperidone*

Canine leishmaniosis is a zoonotic disease caused by Leishmania infantum which is transmited by the bite of a female sandfly to the dog. Its prevention focuses on the use of repellents and immunoprophylaxis. The aim of this study was to investigate the most used preventative measures and its trends over time.

Data on preventaive measures used in client-owned dogs (n=2837) was collected from 73 private veterinary clinics located in Spain (n=60), Italy (n=8), Portugal (n=7) and Cyprus (n=2). Preventative measures were applied in 2595 dogs (91.5%). The most used preventative measure was a repellent alone in 42.7% of the dogs followed by the combination of repellent and vaccination (37.6%), repellent and domperidone (Leisguard®) (12.4%), vaccination alone (4%), repellent, vaccination and domperidone (1.9%), domperidone alone (1%) and vaccination and domperidone (0.3%). Collars were the most used repellents (36.4%) followed by a combination of collar and spot on (33.8%) and spot on alone (29.8%). The Canileish® vaccine (54.7%) was more used than the Letifend® vaccine (45.3%). A significant increase of the use of vaccination and domperidone was detected in 2017 (P=0.001) when compared with the rest of the years studied.

In conclusion, preventative measures against canine leishmaniosis are widely used in highly endemic countries with a preference of repellents alone or in combination with immunoprophylaxis.

# P06

## Competence of the vector restricting tick-borne encephalitis virus spread

Katrin Liebig[1,4], Mathias Boelke[1,4], Gerhard Dobler[2], Lidia Chitimia-Dobler[2,3], Stefanie Becker[1,4]

1 Institute for Parasitology, University of Veterinary Medicine, Hannover, Germany
2 Institute of Microbiology of the Bundeswehr, Munich, Germany
3 Parasitology Unit, Institute of Zoology University of Hohenheim Stuttgart, Germany
4 Research Center for Emerging Infections and Zoonoses, Hannover, Germany

*Keywords: Tick-borne encephalitis virus, vector competence, Ixodes ricinus, in vitro feeding*

Tick-borne encephalitis virus (TBEV), mainly transmitted via the tick vector Ixodes ricinus, causes one of the most important central nervous system (CNS) viral disease, which is endemic in 27 European countries. TBEV endemic foci are usually very restricted and remain stable for extended periods regarding location, prevalence and stability of the viral sequences. To further our understanding of TBEV-tick interactions we used ticks of different areas in Germany (Haselmühl, Hannover, Rauher Busch, Mooshütte) and infected them via an in vitro feeding system. TBEV isolates were obtained from the same endemic foci in Haselmühl, Rauher Busch and Mooshütte. In two experiment series in 2018 and 2019, ticks sampled at the respective TBEV foci showed higher feeding rates (2018, May: Haselmühl 43.81% engorged ticks / Hannover 19.44% and 2019, April: Haselmühl 82.66% / Hannover 53.33%) as well as higher infections rates (2018, October: Haselmühl 26.67% / Hannover 10%). These findings could also been shown in the new TBEV spots Mooshütte and Rauher Busch (2019, April: Mooshütte 64% engorged ticks / Rauher Busch and 45% Mooshütte 28.95% infected ticks / Rauher Busch 4.17%).

Taken together, these findings suggest a specific adaptation of the tick populations to the respective TBEV virus isolate. The resulting vector competence of those populations for TBEV is still unexplored but may explain at least some of the differential distribution of endemic foci.

# P07

## Responses to *Toxoplasma gondii* infection in intestinal organoids from wild rodents and laboratory mice after IFNγ stimulation

Estefania Delgado Betancourt[1], Francesca Torelli[2], Marion Joncic[3], Michael Beekes[3], Christian Klotz[1], Frank Seeber[1]

[1]FG16: Mycotic and Parasitic Agents and Mycobacteria, Robert Koch-Institute, Berlin, Germany
[2] Signalling in Apicomplexan Parasites Laboratory, Francis Crick Institute, London, UK
[3]ZBS 6: Prions and Prion diseases, Robert Koch-Institute, Berlin, Germany

*Keywords: Intestinal organoids, wild rodents, Toxoplasma gondii, Apicomplexa*

After oral infection, the initial encounter of Toxoplasma gondii with its host is at the intestinal epithelium. However, there is limited knowledge about the mechanisms of invasion and the innate cellular responses to the parasite. While laboratory inbred mice have been mostly used for studying these early cellular host responses, wild rodents have not been examined in this respect. However, in Europe vole species like the bank vole Myodes glareolus are more relevant as prey for cats than M. musculus, and are thus important for the transmission of T. gondii to cats, its final host.

To this end we first established and validated a stem cell-derived small intestinal organoid (IO) model from M. glareolus and compared it with IOs from laboratory mice. We tested different medium conditions and determined that maintenance of M. glareolus organoids is most successful under conditions optimized for human IO culture. Moreover, we have evaluated different growth factor combinations to drive differentiation of these organoids into the different cell populations found in the intestine, and identified markers that enables us to characterize them in M. glareolus.

We successfully infected both laboratory M. musculus and M. glareolus IOs with the fast replicating form of T. gondii (tachyzoites) and observed active replication in intestinal epithelial cells, demonstrating the general applicability of this model to study host-parasite interactions. These experiments will be complemented by infections where we stimulate organoids with recombinant interferon gamma from M. glareolus (recMgIFNγ) and M. musculus (mIFNγ), respectively, and evaluate its effect on parasite growth and replication.

# P08

## Exploring the relationship between susceptibility to canine leishmaniosis and anti-P. perniciosus saliva antibodies in Ibizan hounds and dogs of other breeds

Alexis Burnham[1], M. Magdalena Alcover Amengual[2], Laura Ordeix[1], Laura Willen[3], Tatiana Kostalova[3], Petr Volf[3], Laia Solano-Gallego[1]

[1] Universitat Autònoma de Barcelona
[2] Universitat de Barcelona
[3] Charles University of Prague

*Keywords: Canine leishmaniosis, Leishmania infantum, Phlebotomus perniciosus, Ibizan hounds, anti-saliva antibodies*

Canine leishmaniosis from Leishmania infantum is a neglected zoonosis transmitted by sand flies like Phlebotomus perniciosus. Clinical signs and susceptibility vary by host immune response. Ibizan hounds are more resistant and show higher prevalence of L. infantum papular dermatitis, linked with a parasite specific cellular immune response. Immunocompetence could be attributed to more frequent exposure to sand flies, eliciting an anti-saliva antibody response in dogs. We examined anti-P. perniciosus saliva IgG in 47 Ibizan hounds and 45 other breed dogs in Mallorca using 3 methods: P. perniciosus whole salivary gland homogenate (SGH) ELISA, rSP03B ELISA, and rSP03B rapid test. Antibody levels were correlated with clinical, immunological, and parasitological parameters. Additionally, diagnostic performance was evaluated between methods. Results indicate significantly higher SGH ELISA units in Ibizan hounds than other breeds ($p=0.0061$). Dogs with papular dermatitis displayed greater SGH and rSPO3B ELISA units, but differences were not statistically significant ($p=0.2980$; $p=0.1554$). Older age and L. infantum seropositivity were also considered significant factors in antibody levels according to at least one test. Fair agreement was found between all three tests, with the highest value between SGH and rSP03B ELISA. Additional sampling is needed to confirm results, but anti-P. perniciosus saliva IgG appear to negatively correlate with susceptibility to L. infantum infection.

# P09

## Water-borne protozoan in free-ranging raccoons (Procyon lotor) from Leipzig metropolitan area, Germany

Zaida Rentería-Solís[1], Stefan Birka[2], Franziska Ackermann[1,3], Nina Król[4], Anna Obiegala[4]

[1]Institute for Parasitology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
[2]Institute for Food Hygiene, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
[3]Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
[4]Institute for Animal Hygiene and Public Health, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

*Keywords: Cryptosporidium, Giardia, raccoon, zoonoses, protozoa*

The raccoon is an introduced species in Europe since 1934. Still, there is a lack of information in infectious pathogens present in these animals. Raccoons have not only a natural preference for water bodies but also the ability to benefit from anthropogenic resources. Therefore, they could play a role in the ecology and epidemiology of some water-borne pathogens. Cryptosporidium spp. and Giardia spp. are both ubiquitous zoonotic protozoa commonly found in water bodies. Both of them cause intestinal disease in a large variety of hosts, including humans. The objective of this study is to elucidate the presence of both Cryptosporidium spp. and Giardia spp. in free-ranging raccoons collected from Leipzig metropolitan area, Saxony, Germany. Hunted raccoons were collected (n=40). A full necropsy was performed in each animal. Intestine as well as faecal samples were taken for further examination. DNA was extracted from faeces using a commercial kit. Cryptosporidium spp. was confirmed after successful amplification of an 820 bp nested PCR product of the SSU rRNA fragment. Similarly, a nested PCR assay was conducted for Giardia spp. Overall, Cryptosporidium spp. was confirmed exclusively in one animal and Giardia spp. was detected in 3 individuals. Genotyping and phylogenetic analysis of both parasites will be performed. This is the first report of Cryptosporidium spp. and Giardia spp. in raccoons in Germany highlighting raccoons as possible vectors of infectious diseases.

# Poster Presentations Next Generation Sequencing

## S01 – S04

# S01

## GenoSalmSurv – An Integrated Genome-based surveillance system for Salmonella

Sandra Simon[1], Laura Uelze[2], Michael Pietsch[3], Andreas Sing[4], Antje Flieger[3], Ulrich Busch[5], Burkhard Malorny[2]

[1] Robert Koch-Institut
[2] Bundesinstitut für Risikobewertung
[3] Robert Koch Institut
[4] Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit
[5] Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit

*Salmonella, surveillance, Whole-Genome Sequencing, Next-Generation Sequencing*

In Germany, Salmonellosis is the second most frequently reported bacterial diarrheal disease with high hospitalization rates. Zoonotic Salmonella spp. cause many regional and international food-borne disease outbreaks, which are of great concern for public health and an economic risk factor for the food industry.
Next Generation Sequencing (NGS) is a high-throughput method with maximum resolution, and as such the optimal tool for rapid and accurate pathogen monitoring and infection source analysis. However, an effective implementation of NGS methods has been hampered by the lack of standardized methods, uniform quality criteria and data sharing strategies, all of which are mandatory for a successful interpretation of sequencing data from different sources.
To overcome this challenge, the GenoSalmSurv project aims to establish a working model towards an integrated genome-based surveillance system of Salmonella spp. Backbone of the model is the harmonization of laboratory procedures and sequencing protocols; the implementation of open-source bioinformatics tools for data analysis; and the establishment of routine practices for data sharing for a uniform result interpretation. Furthermore authorities and multipliers will be surveyed, consulted and trained with the aim to make genome-based surveillance and outbreak analyses easily accessible to all authorities involved and thus to accelerate and widely implement its establishment across all sectors.

# S02

## The interplay of respiratory infections, sociality and microbial community composition in wild chimpanzees in the Taï National Park (TNP), Côte d'Ivoire

Markus Ulrich, Jan Gogarten, Josefine Hayeß, Roman Wittig, Sebastien Calvignac-Spencer, Fabian Leendertz

[1] Robert Koch - Institut
[2] Max Planck Institut für evolutionäre Anthropologie

*Keywords: Non-human primates, microbiota, infectious diseases*

The wild chimpanzee communities in Taï National Park (TNP), Côte d'Ivoire, were affected by several disease outbreaks (HRSV, HMPV, *S. pneumoniae*) in the years 2004 and 2006. Here we want to test the hypothesis that such outbreaks affect social structures and result in a shift of microbial community composition on the group-level. To do this, we investigate microbial community composition before and after these outbreaks and compare these data with changes in the social structure of the affected groups. We analyze fecal nucleic acids and use an amplicon sequencing approach to describe gut microbiota (bacteria and archaea), virus and eukaryotic parasite communities and shotgun sequencing for the identification of phages. As previous studies showed that the microbiota is affected by diet and seasonality, we try to control for diet effects with the incorporation of molecular diet analysis and for seasonality by the use of remote sensing data. For the data analysis we integrate social data (grooming, aggression, group composition), environmental data (precipitation, food availability, vegetation indices, territory size), diet (mammals, plants and insects), and microbiota composition (bacteria, archaea, eukaryotic parasites, phages, and viruses). We will present a first set of analyses during the International Symposium on Zoonosis 2019.

# S03

## Whole genome sequencing of two novel strains of *Coxiella burnetii* isolated from goat and sheep in Germany

Dimitrios Frangoulidis[1], Akinyemi Fasemore[2], Mathias Walter[1], Konrad Förstner[3], Thomas Dandekar[4]

[1] Bundeswehr
[2] Bundeswehr, University of Cologne
[3] University of Cologne
[4] University of Würzburg

*Keywords: Q fever, Coxiella burnetii, whole genome sequencing, outbreaks, small ruminants*

Coxiella burnetii, the causative bacterial agent of the zoonotic worldwide-distributed disease Q fever can infect ruminants and humans. Persistence in German small ruminants is the source of periodic human Q fever infections and outbreaks. One mission of the Q-GAPS consortium is to enlarge the genomic background knowledge about Coxiella in Germany including generating reliable molecular epidemiological data. Therefore, material from infected flocks was collected, isolated and sequenced. Here we present two novel whole genome sequences of C. burnetii from flock outbreaks in 2018 in Schleswig-Holstein (goats) and Baden-Württemberg (sheep).
In the past only three German strains were sequenced: two sheep isolates from 1991 and 1994 and one cattle strain from 2003. DNA of the new strains was sequenced on an Illumina MiSeq instrument followed by a genome assembly using SPAdes v3.12 leading to 68 contigs with a total sequence length of 2,000,714 bp for the strain "TiHoQ-2219" and 38 contigs with total sequence length of 2,039,178 bp for the strain "TiHoQ-1091". Average Nucleotide Identity (ANI) was used to compare both genomes with others published in RefSeq. Interestingly, a first analysis showed relationship to a cattle strain from the Netherlands isolated in 2009.
The preliminary results presented here will be extended by further analyses including the evaluation of IS elements and tandem repeats.

# S04

## Genomic features and antimicrobial resistance of *P. aeruginosa* from animals

Franziska Dambon[1], Lisa Jacobmeyer[1], Ellen Prenger-Berninghoff[1], Ursula Leidner[1], Ivonne Stamm[2], Stephan Göttig[3], Torsten Semmler[4], Christa Ewers[1]

[1] Institute of Hygiene and Infectious Diseases of Animals, Giessen, Germany
[2] IDEXX GmbH, Ludwigsburg, Germany
[3] Institute of Medical Microbiology and Infection Control, Hospital of the Johann Wolfgang von Goethe University, Frankfurt am Main
[4] Robert Koch Institute, Microbial Genomics, Berlin, Germany

*Keywords: Pseudomonas aeruginosa, animal, whole genome sequencing*

Pseudomonas (P.) aeruginosa is a leading cause of hospital-acquired infections worldwide and is also involved in animal infections. The genomic background and resistance features of animals strains and overlaps with humans strains are hardly explores. We sequenced the genome of animal (cats/dogs (n69); livestock (n=3); others (n=5) from ear (n=21); urine (n=19); wound (n=27); other (n=10)) and human (n=29) clinical strains and tested their antimicrobial susceptibility (VITEK 2 (AST-GN38; EUCAST breakpoints)). NGS data were used to determine sequence types, serotypes, resistance and virulence genes. A maximum-likelihood phylogeny was constructed by including P. aeruginosa genomes from the public database.Thirteen (16.9%) animal strains were classified as 3/4-MRGN, imipenem resistance was due to mutation/loss of oprD; acquired carbapenemases were not detected. We identified several human-related STs, including high-risk clones ST244/ST395 that strains clustered close to human strains. Also serotypes resembled those of human isolates, e.g. O11 (14.9%), which is prevalent among nosocomial outbreaks and O6 (23.9%). As in human medicine, our O11 strains were associated with the highly relevant cytotoxin exoU (90%), while O6 strains were linked to the less cytotoxic exoS (87.5%).The finding of high-risk clones and multidrug-resistance among animal strains indicated significant overlaps between animal and human P. aeruginosa strains, suggesting putative interspecies transmission.

# Participants

| Name | | Affiliation and Email address |
|---|---|---|
| Eric | Agboli | Bernhard Nocht Institute for Tropical Medicine;<br>agboli@bnitm.de |
| Abdollah | Aghebatrafat | Robert-Koch Institut;<br>aghebatrafata@rki.de |
| Sascha | Al Dahouk | German Federal Institute for Risk Assessment;<br>sascha.al-dahouk@bfr.bund.de |
| Valerie | Allendorf | Friedrich-Loeffler-Institut;<br>valerie.allendorf@fli.de |
| Alejandra | Álvarez Fernández | Universidad Autonoma de Barcelona;<br>alejandra.alvarez@uab.cat |
| Julia | Assmann | Robert Koch-Institut;<br>assmannj@rki.de |
| David | Attuy Vey da Silva | Federal Institute for Risk Assessment, BfR;<br>silvadav@usp.br |
| Steffen | Backert | Friedrich-Alexander Universität Erlangen-Nürnberg;<br>steffen.backert@fau.de |
| Sara | Balk | Friedrich-Loeffler-Institut Jena;<br>sara.balk@fli.de |
| Laura Andrea | Barrero Guevara | Freie Universität Berlin;<br>la.barrero40@gmail.com |
| Ben | Bauer | Stiftung Tierärztliche Hochschule Hannover;<br>benjamin.bauer@tiho-hannover.de |
| Marta | Baxarias | Universitat Autònoma de Barcelona;<br>marta.baxarias@uab.cat |
| Dietmar | Becher | MICROMUN GmbH;<br>becher@micromun.de |
| Stefanie | Becker | Institute for Parasitology;<br>stefanie.becker@tiho-hannover.de |
| Racem | Ben Romdhane | Institut für Veterinär-Epidemiologie und Biometrie - Freie Universität Berlin;<br>ben.romdhane.racem@fu-berlin.de |
| Stefan | Bereswill | Charité-Institut für Mikrobiologie und Infektionsimmunologie;<br>hi.bereswill@t-online.de |
| Max | Berlin | Nationales Konsiliarlabor für Dermatophyten;<br>max.berlin@charite.de |
| Malena | Bestehorn-Willmann | University of Hohenheim;<br>MalenaBestehornWillmann@bundeswehr.org |
| Astrid | Bethe | FU Berlin;<br>astrid.bethe@fu-berlin.de |
| Nadja | Bier | German Federal Institute for Risk Assessment;<br>nadja.bier@bfr.bund.de |
| Florian | Binder | Friedrich-Loeffler-Institut;<br>florian.binder@fli.de |
| Tobias | Bleicker | Institute of Virology - Charité Universitaetsmedizin Berlin;<br>tobias.bleicker@charite.de |
| Katharina | Bobe | Ceva Innovation Center GmbH; |

| | | |
|---|---|---|
| | | katharina.bobe@idt-biologika.de |
| Mathias | Boelke | Stiftung Tierärztliche Hochschule Hannover;<br>mathias.boelke@tiho-hannover.de |
| Marcel | Bokelmann | Robert Koch Institute;<br>bokelmannm@rki.de |
| Katrin | Bote | Institute for Animal Hygiene and Environmental Health;<br>katrin.bote@fu-berlin.de |
| Veronika | Breitkopf | Tierärztliche Hochschule Hannover;<br>veronika.breitkopf@tiho-hannover.de |
| Stefan | Brockmann | Kreisgesundheitsamt Reutlingen;<br>s.brockmann@kreis-reutlingen.de |
| Stefan | Brockmann | Landesgesundheitsamt Baden-Württemberg;<br>stefan.brockmann@rps.bwl.de |
| Vanessa | Brückner | Institute of Food Safety and Food Hygiene, FU Berlin;<br>vanessa.brueckner@fu-berlin.de |
| Sarah | Brüggemann-Schwarze | Freie Universität Berlin, Institut für Geflügelkrankheiten;<br>sarah.brueggemann@fu-berlin.de |
| Alexis | Burnham | Universitat Autònoma de Barcelona;<br>burnham.alexis@gmail.com |
| Sten | Calvelage | Friedrich-Loeffer-Institut;<br>sten.calvelage@fli.de |
| Friedrich | Chasin | EVIS GmbH;<br>chasin@evis-solutions.de |
| Xyomara | Chavez Pacheco | None;<br>x.chavezpacheco@gmail.com |
| Lidia | Chitimia-Dobler | Bundeswehr Institute of Microbiology;<br>lydiachitimia@gmail.com |
| Céline | Christiansen | Robert Koch Institut;<br>christiansenc@rki.de |
| Nicole | Cichon | German;<br>nicole.cichon@fli.de |
| Filip | Cierniak | FLI;<br>filip.cierniak@fli.de |
| Arne | Cordsmeier | Universitätsklinikum Erlangen Institut für Mikrobiologie, Immunologie und Hygiene;<br>arne.cordsmeier@uk-erlangen.de |
| Victor | Corman | Institute of Virology, Charite - Universitätsmedizin Berlin;<br>victor.corman@charite.de |
| Constanze | Cox | Robert Koch-Institut;<br>coxc@rki.de |
| Katrin | Dähre | BVL;<br>katrin.daehre@bvl.bund.de |
| Franziska | Dambon | Institute of Hygiene and Infectious Diseases of Animals;<br>jan.a.dambon@gmail.com |
| Larissa | Dangel | Landesgesundheitsamt Baden-Württemberg;<br>larissa.dangel@rps.bwl.de |
| Walter | Däubener | Medizinische Mikrobiologie und Krankenhaushygiene; |

| | | |
|---|---|---|
| | | daeubene@uni-duesseldorf.de |
| Nicole | de Buhr | University of Veterinary Medicine Hannover;<br>ndebuhr@tiho-hannover.de |
| Katja | Deeg | Bundesanstalt für Landwirtschaft und Ernährung;<br>katja.deeg@ble.de |
| Estefania | Delgado Betancourt | Robert Koch Institut;<br>delgado-betancourte@rki.de |
| Gunnar | Demuth | Independent;<br>g.demuth@berlin.de |
| Gerhard | Dobler | Bundeswehr Institute of Microbiology;<br>gerharddobler@bundeswehr.org |
| Monika | Drážovská | University of veterinary medicine and pharmacy in Košice;<br>drazovska@gmail.com |
| Johannes | Dreesman | Niedersächsisches Landesgesundheitsamt;<br>johannes.dreesman@nlga.niedersachsen.de |
| Marco | Drehmann | University of Hohenheim, Department Parasitology;<br>marco.drehmann@uni-hohenheim.de |
| Stephan | Drewes | Friedrich-Loeffler-Institut;<br>stephan.drewes@fli.de |
| Anou | Dreyfus | Medical Department, Swiss Tropical and Public Health Institute;<br>anou.dreyfus@swisstph.ch |
| Christian | Drosten | Charité - Universitätsmedizin Berlin;<br>christian.drosten@charite.de |
| Ariane | Düx | Robert Koch-Institut;<br>duexa@rki.de |
| Janine | Dzierzon | FU Berlin, Institute of Food Saftey and Food Hygiene, Working Group Meat Hygiene;<br>janine.dzierzon@fu-berlin.de |
| Marco | Ebert | Hochschule Neubrandenburg;<br>eber@hs-nb.de |
| Arnt | Ebinger | Friedrich-Loeffler-Institut, Institut für Virusdiagnostik;<br>arnt.ebinger@fli.de |
| Natalie | Effelsberg | Institute of Hygiene, University Hospital Münster;<br>natalie.effelsberg@ukmuenster.de |
| Daniel | Eibach | Bernhard Nocht Institute for Tropical Medicine;<br>eibach@bnitm.de |
| Philip | El-Duah | Institute of Virology: Charité – Universitätsmedizin Berlin;<br>philip.el-duah@charite.de |
| Heinz | Ellerbrok | Robert Koch Institut;<br>ellerbrokh@rki.de |
| Carmen | Embregts | Erasmus MC - Department of Viroscience;<br>c.embregts@erasmusmc.nl |
| Duygu | Emirhar | German Federal Institute for Risk Assessment;<br>duygu.emirhar@bfr.bund.de |
| Lennard | Epping | Robert Koch Institute;<br>eppingl@rki.de |
| Benedikt | Fabian | Robert Koch-Institute; |

| | | |
|---|---|---|
| | | fabianbe@rki.de |
| Katrin | Fachet | University of Hohenheim, Department of Parasitology;<br>fachet@uni-hohenheim.de |
| Alexander | Falkenhagen | The German Federal Institute for Risk Assessment;<br>alexander.falkenhagen@bfr.bund.de |
| Akinyemi Mandela | Fasemore | Bundeswehr Institute of Microbiology;<br>akinyemi.fasemore@stud-mail.uni-wuerzburg.de |
| Ulrike | Felgenhauer | JLU Giessen;<br>ulrike.felgenhauer@vetmed.uni-giessen.de |
| Shari | Fell | LGL Oberschleißheim;<br>shari.fell@web.de |
| Marta | Ferrandis Vila | Friedrich-Loeffler-Institut;<br>marta.ferrandisvila@fli.de |
| Edmilson | Ferreira de Oliveira Filho | Charite University Hospital;<br>edmilson.oliveira@charite.de |
| Stefan | Finke | Friedrich-Loeffler-Institut;<br>stefan.finke@fli.de |
| Berenice | Fischer | Infection biology unit Deutsches Primatenzentrum GmbH;<br>berenice.fischer@gmx.de |
| Carlo | Fischer | Insittut für Virologie, Charité - Universitätsmedizin Berlin;<br>carlo.fischer@charite.de |
| Martin | Fischer | Robert Koch Institut;<br>fischerm@rki.de |
| Silke | Fischer | Landesgesundheitsamt Baden-Württemberg;<br>silke.fischer@rps.bwl.de |
| Sina | Fischer | Chemisches und Veterinäruntersuchungsamt Karlsruhe;<br>sina.fischer@cvuaka.bwl.de |
| Susanne | Fleischmann | Freie Universität Berlin; Institute of Food Safety and Food Hygiene;<br>susanne.fleischmann@fu-berlin.de |
| Judith | Flügge | Institute for Tropical Medicine;<br>judith.fluegge@uni-tuebingen.de |
| Greta | Flüh | Uniklinikum Düsseldorf, Insitut für medizinische Mikrobiologie;<br>greta.flueh@med.uni-duesseldorf.de |
| Dominik | Forster | LMU;<br>dominik.forster@micro.vetmed.uni-muenchen.de |
| Cara | Förster | Tierärztliche Hochschule Hannover;<br>cara.foerster@tiho-hannover.de |
| Dimitrios | Frangoulidis | Bundeswehr;<br>dimitriosfrangoulidis@instmikrobiobw.de |
| Jonas | Fuchs | Institute of Virology, Medical Center, Faculty of Medicine, University Freiburg, Germany;<br>jonas.fuchs@uniklinik-freiburg.de |
| Enrico | Georgi | Bundeswehr Institute of Microbiology;<br>enricogeorgi@web.de |
| Corine | Geurts van Kessel | Erasmus MC;<br>c.geurtsvankessel@erasmusmc.nl |
| Christine | Goffinet | Charité - Universitätsmedizin Berlin; |

| | | |
|---|---|---|
| | | christine.goffinet@charite.de |
| Jan | Gogarten | Robert Koch Institute;<br>jan.gogarten@gmail.com |
| Julia | Golz | Bundesinstitut für Risikobewertung;<br>julia.golz@bfr.bund.de |
| Annika | Graaf | Friedrich-Loeffler-Institute, Institute of Diagnostic Virology;<br>annika.graaf@fli.de |
| Tobias | Gräßle | Robert Koch-Institut;<br>graesslet@rki.de |
| Mirjam | Grobbel | Bundesinstitut für Risikobewertung;<br>mirjam.grobbel@bfr.bund.de |
| Martin H. | Groschup | Friedrich-Loeffler-Institut Bundesforschungsinstitut für Tiergesundheit;<br>martin.groschup@fli.de |
| Katharina | Gruchenberg | TÜV SÜD Product Service GmbH;<br>katharina.gruchenberg@tuev-sued.de |
| Taras | Günther | Freie Universität zu Berlin;<br>guentert@zedat.fu-berlin.de |
| Frederic | Gusmag | Tierärztliche Hochschule Hannover - Insitut für Parasitologie;<br>frederic.gusmag@tiho-hannover.de |
| Jan-Dirk | Häger | Institut für molekulare Pathogenese (IMP), Friedrich-Löffler-Institut, Jena;<br>jandirkhaeger@aol.de |
| Jens | Hammerl | German Federal Institute for Risk Assessment;<br>jens-andre.hammerl@bfr.bund.de |
| Birgit | Hargens | Bundeswehr;<br>bhargens@yahoo.de |
| Dominik | Harms | Robert Koch-Institut;<br>dominik.harms@yahoo.de |
| Julia | Hartlaub | Friedrich-Loeffler-Institut;<br>julia.hartlaub@fli.de |
| Carolin | Hattendorf | Bernhard-Nocht-Institut für Tropenmidizin;<br>carolin.hattendorf@bnitm.de |
| Kristin | Heenemann | University of Leipzig, Faculty of Veterinary Medicine, Institute of Virology;<br>kristin.heenemann@vetmed.uni-leipzig.de |
| Markus | Heimesaat | Charité-University Medicine Berlin; Institute for Microbiology, Immunology and Infectious Diseases;<br>markus.heimesaat@charite.de |
| Mareike | Heinig | Tierärztliche Hochschule Hannover;<br>mareike.heinig@tiho-hannover.de |
| Janine | Heise | German Federal Institute for Risk Assessment;<br>janine.heise@bfr.bund.de |
| Andrea | Helbich | Institut für Mikrobiologie der Bundeswehr;<br>andreahelbich@bundeswehr.org |
| Christiane | Herden | Justus-Liebig-University Gießen;<br>christiane.herden@vetmed.uni-giessen.de |
| Kyra | Hermanns | Institute of Virology, Charité - Universitätsmedizin Berlin;<br>kyra.hermanns@charite.de |

| | | |
|---|---|---|
| Luis Pablo | Hervé Claude | Universidad de Chile;<br>lpherve@u.uchile.cl |
| Elisa | Heuser | Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases;<br>elisa.heuser@fli.de |
| Antonia | Hilbig | Robert-Koch-Institute F. Leendertz /Charité Berlin;<br>hilbiga@rki.de |
| Katja | Hille | University of Veterinary Medicine Hannover;<br>katja.hille@tiho-hannover.de |
| Chris | Hoffmann | Bernhard-Nocht-Institut für Tropenmedizin;<br>hoffmann@bnitm.de |
| Donata | Hoffmann | Friedrich-Loeffler-Institut;<br>donata.hoffmann@fli.de |
| Markus | Hoffmann | Deutsches Primatenzentrum GmbH - Leibniz Institut für Primatenforschung;<br>mhoffmann@dpz.eu |
| Constanze | Hoffmann-Dechamps | Institut Pasteur;<br>con.hoffmann@gmail.com |
| Dirk | Hofreuter | Federal Institute for Risk Assessment, Department of Biological Safety;<br>dirk.hofreuter@bfr.bund.de |
| Hannah | Höglund-Braun | Heinrich-Heine Universität Düsseldorf;<br>hoeglundbraun@gmail.com |
| Cora Marielle | Holicki | Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut;<br>cora.holicki@fli.de |
| Katharina | Hommerich | University of Veterinary Medicine Hannover;<br>katharina.hommerich@tiho-hannover.de |
| Charlotte | Huber | Robert Koch Institute;<br>huberc@rki.de |
| Sarah | Hüttl | University of Veterinary Medicine Hannover;<br>sarah.huettl@tiho-hannover.de |
| Alexandra | Irrgang | Bundesinstitut für Risikobewertung;<br>alexandra.irrgang@bfr.bund.de |
| Claudia | Jäckel | German Federal Institute for Risk Assessment;<br>claudia.jaeckel@bfr.bund.de |
| Anna | Jackova | University of Veterinary Medicine and Pharmacy in Kosice;<br>anna.jackova@uvlf.sk |
| Jens | Jacob | Julius Kühn-Institut (JKI) Federal Research Centre for Cultivated Plants, Vertebrate Research;<br>jens.jacob@julius-kuehn.de |
| Tariq | Jamil | Friedrich Loeffler Instit;<br>tariq.jamil@fli.de |
| Friederike | Jansen | Institut für Virologie (IVM);<br>friederike.jansen@ukmuenster.de |
| Stephanie | Jansen | BNITM;<br>jansen@bnitm.de |
| Kathrin | Jeske | Friedrich-Loeffler-Institut;<br>kathrin.jeske@fli.de |
| Vanessa | Johanns | Robert Koch-Institut; |

| | | |
|---|---|---|
| | | johannsv@rki.de |
| Reimar | Johne | Bundesinstitut für Risikobewertung;<br>reimar.johne@bfr.bund.de |
| Sandra | Junglen | Charité Universitätsmedizin Berlin;<br>sandra.junglen@charite.de |
| Barbara | Junker | DLR-PT;<br>barbara.junker@dlr.de |
| Katharina | Juraschek | PhD Student;<br>katharina.juraschek@bfr.bund.de |
| Franziska | Kaiser | franziska.kaiser@tiho-hannover.de;<br>franziska.kaiser@tiho-hannover.de |
| Svetlana | Kasabova | University for Veterinary Medicine, Hannover, Germany;<br>svetlana.kasabova@tiho-hannover.de |
| Anne | Kauter | Robert Koch-Istitut;<br>kautera@rki.de |
| Sophie | Kittler | Research Center for Emerging Infections and Zoonoses;<br>sophie.kittler@tiho-hannover.de |
| Silke | Klee | Robert Koch-Institut;<br>klees@rki.de |
| Antonia | Klein | Friedrich-Loeffler-Institut;<br>antonia.klein@fli.de |
| Hannah | Kleine-Weber | PhD Student;<br>hkleine-weber@dpz.eu |
| Zinaida | Klestova | State Scientific-Control Institute of Biotechnology and Strains of Microorganisms;<br>zinaklestova@gmail.com |
| Christian | Klotz | Robert Koch-Institute;<br>klotzc@rki.de |
| Michael | Knittler | Friedrich-Loeffler-Institut (FLI), Institute of Immunology;<br>michael.knittler@fli.de |
| Robert | Koban | Robert Koch-Institut;<br>kobanr@rki.de |
| Iris | Kobusch | Fachhochschule Südwestfalen;<br>kobusch.iris@fh-swf.de |
| Philipp | Koch | Julius Kühn-Institut;<br>philipp.koch@julius-kuehn.de |
| Robin | Koeck | DRK Kliniken Berlin;<br>r.koeck@drk-kliniken-berlin.de |
| Wiebke | Köhl | BioDoc;<br>wiebke.koehl@gmx.de |
| Anne | Kontek | LAVES Niedersachsen, Lebensmittel- und Veterinärinstitut Oldenburg;<br>anne.kontek@laves.niedersachsen.de |
| Anne | Kopp | Institute of Virology, Charité - Universitätsmedizin Berlin;<br>anne.kopp@charite.de |
| Peter | Kopp | IDEXX VetMedLabor GmbH;<br>peter-kopp@idexx.com |
| Ursula | Kopp | DLR Projektträger; |

| | | |
|---|---|---|
| | | ursula.kopp@dlr.de |
| Sophia | Körner | Friedrich-Loeffler-Institut;<br>sophia.koerner@fli.de |
| Isabelle | Kramer | Goethe University Frankfurt;<br>isabelle_kramer@gmx.de |
| Lothar | Kreienbrock | University of Veterinary Medicine Hannover;<br>lothar.kreienbrock@tiho-hannover.de |
| Teresa | Kreusch | Robert Koch Institute;<br>kreuscht@rki.de |
| Jennifer | Krieg | Landesgesundheitsamt Stuttgart;<br>krieg.jenny@gmx.de |
| Nina | Król | Institut für Tierhygiene und Öffentliches Veterinärwesen, Universität Leipzig;<br>nina.krol@vetmed.uni-leipzig.de |
| Henrike | Krüger | Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin;<br>henrike.krueger@fu-berlin.de |
| Karolin | Krüger | Federal Office of Consumer Protection and Food Safety;<br>karolin.krueger@bvl.bund.de |
| Nadine | Krüger | Institut of Virology, University of Veterinary Medicine Hannover;<br>nadine.krueger@tiho-hannover.de |
| Ulrich | Kuch | Goethe-Universität Frankfurt;<br>kuch@med.uni-frankfurt.de |
| Bernd | Kurlemann | Private;<br>antonius.hartanto@hotmail.com |
| Andreas | Kurth | Robert Koch Institute;<br>kurtha@rki.de |
| Susanne | Kutzora | Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit;<br>susanne.kutzora@lgl.bayern.de |
| Mayke | Leggewie | Bernhard Nocht Institute for Tropical Medicine;<br>leggewie@bnitm.de |
| Katrin | Liebig | Tierärztliche Hochschule Hannover;<br>katrin.liebig@tiho-hannover.de |
| Tobias | Lienen | German Federal Institute for Risk Assessment;<br>tobias.lienen@bfr.bund.de |
| Alexander | Lindau | University of Hohenheim, Department Parasitology;<br>alexander.lindau@uni-hohenheim.de |
| Sabrina | Linnemann | Fachhochschule Südwestfalen;<br>linnemann.sabrina@fh-swf.de |
| Therese | Löhrich | WWF;<br>therese_loehrich@yahoo.de |
| Therese | Löhrich | RKI;<br>tloehrich@wwfcar.org |
| Nelly Sophie | Lönker | Georg-August-Universität Göttingen;<br>n.loenker@stud.uni-goettingen.de |
| Antina | Lübke-Becker | FU Berlin, Institut für Mikrobiologie und Tierseuchen;<br>antina.luebke-becker@fu-berlin.de |

| | | |
|---|---|---|
| Stephan | Ludwig | Institut für Virologie (IVM);<br>ludwigs@uni-muenster.de |
| Renke | Lühken | Bernhard Nocht Institute for Tropical Medicine;<br>renkeluhken@gmail.com |
| Anja | Lührmann | Universitätsklinikum Erlangen;<br>anja.luehrmann@uk-erlangen.de |
| Dörte | Lüschow | Institute of Poultry Diseases, Freie Universität Berlin;<br>doerte.lueschow@fu-berlin.de |
| Stefanie | Lüth | German Federal Institute for Risk Assessment;<br>stefanie.lueth@bfr.bund.de |
| Christin | Mache | Robert Koch-Institute;<br>ch_mache@gmx.de |
| Ute | Mackenstedt | University of Hohenheim;<br>mackenst@uni-hohenheim.de |
| Olga | Makarova | Freie Universität Berlin;<br>olga.makarova@fu-berlin.de |
| Yue | Ma-Lauer | Max von Pettenkofer Institute;<br>ma_lauer@mvp.uni-muenchen.de |
| Burkhard | Malorny | German Federal Institute for Risk Assessment;<br>burkhard.malorny@bfr.bund.de |
| Marco | Marklewitz | Charité-Universitätsmedizin Berlin, Berlin, Germany;<br>marco.marklewitz@charite.de |
| Svea | Matthiesen | German;<br>svea.matthiesen@fli.de |
| Michael | Mauermeir | Institute of Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen;<br>michael.mauermeir@fau.de |
| Sven | Maurischat | German Federal Institute for Risk Assessment, Unit Biological Safety;<br>sven.maurischat@bfr.bund.de |
| Deborah | Maus | Robert Koch Institute;<br>mausd@rki.de |
| Anne | Mayer-Scholl | Federal Institute for Risk Assessment;<br>anne.mayer-scholl@bfr.bund.de |
| Kristina | Meier | Bernhard-Nocht-Institut;<br>kristina.meier@bnitm.de |
| Christian | Menge | Friedrich-Loeffler-Institut;<br>christian.menge@fli.de |
| Teresa | Merk | german;<br>resa288@gmx.de |
| Roswitha | Merle | Freie Universität Berlin, Institute for Veterinary Epidemiology and Biostatistics;<br>roswitha.merle@fu-berlin.de |
| Katja | Mertens-Scholz | Friedrich-Loeffler-Institut;<br>katja.mertens-scholz@fli.de |
| Octavio | Mesa-Varona | BfR;<br>octavio.mesa-varona@bfr.bund.de |
| Leontina | Milea | Tierärztin Landkreis Prignitz;<br>marutza85@yahoo.com |

| | | |
|---|---|---|
| Nina | Militzer | Institute for Parasitology and Tropical Veterinary Medicine;<br>nina.militzer@fu-berlin.de |
| Amira | Moawad | Friedrich Loeffler Institute;<br>amira.moawad@fli.de |
| Gerzon | Motola | Institut für Geflügelkrankheiten;<br>gerzon.motola@fu-berlin.de |
| Soraya | Mousavi | Charite - Universitätsmedizin Berlin;<br>soraya.mousavi@charite.de |
| Benjamin | Mubemba | Robert Koch-Institut;<br>mubembab85@yahoo.co.uk |
| Michael | Muehlebach | Paul-Ehrlich-Institut;<br>michael.muehlebach@pei.de |
| Hannah | Müller | Fachhochschule Südwestfalen;<br>mueller.hannah@fh-swf.de |
| Jana | Müller | Justus Liebig Universität Gießen;<br>jana.mueller@vetmed.uni-giessen.de |
| Janina | Müller | Institut für Veterinär-Pathologie, Gießen;<br>janina.mueller@vetmed.uni-giessen.de |
| Frauke | Müller | Bundesanstalt für Landwirtschaft und Ernährung;<br>frauke.mueller@ble.de |
| Marcel | Müller | Charité- Universitätsmedizin Berlin;<br>marcel.mueller@charite.de |
| Aemero | Muluneh | Veterinarian;<br>aemero.muluneh@lua.sms.sachsen.de |
| Doreen | Muth | LAGeSo Berlin;<br>doreen.muth@lageso.berlin.de |
| Amir | Nakar | Fridrich Schiller University;<br>amir.nakar@uni-jena.de |
| Lisa Hanne | Nau | Institut für Tierhygiene und Öffentliches Veterinärwesen, Universität Leipzig;<br>l.nau@studserv.uni-leipzig.de |
| Jessica | Neid | Goethe-Universität Frankfurt;<br>jessica.neid@googlemail.com |
| Kerstin | Neubert | Freie Universität Berlin;<br>kerstin.neubert@fu-berlin.de |
| Markus | Neumann | Robert Koch-Institut;<br>1.markusneumann@web.de |
| Daniela | Niemeyer | Charité - Universitätsmedizin Berlin, Institute of Virology;<br>daniela.niemeyer@charite.de |
| Daniel | Nobach | Justus-Liebig-University Giessen;<br>daniel.nobach@vetmed.uni-giessen.de |
| Anna | Obiegala | Institut für Tierhygiene, Universität Leipzig;<br>anna.obiegala@vetmed.uni-leipzig.de |
| Rainer | Oehme | State Health Office Baden-Württemberg;<br>rainer.oehme@rps.bwl.de |
| Lisa | Oestereich | Bernhard-Nocht-Institut für Tropenmedizin;<br>oestereich@bnitm.de |

| | | |
|---|---|---|
| Silke | Olschewski | Bernhard Nocht Institute for Tropical Medicine;<br>olschewski@bnitm.de |
| Anna | Ondrejková | University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic;<br>anna.ondrejkova@uvlf.sk |
| Susann | Opherden | Freie Universität Berlin, Institut für Lebensmittelsicherheit und -hygiene;<br>opherden@zedat.fu-berlin.de |
| Annette | Orellana | LAVES, Lebensmittel- und Veterinärinstitut Oldenburg;<br>annette.orellana@laves.niedersachsen.de |
| Verena | Oswaldi | FU Berlin;<br>verena.oswaldi@fu-berlin.de |
| Dennis | Pägelow | Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin;<br>dennis.paegelow@fu-berlin.de |
| Sofia | Paraskevopoulou | Institute of Virology, Charité Universitätsmedizin Berlin;<br>sofia.paraskevopoulou@charite.de |
| Livia | Patrono | Robert Koch Institute;<br>patronol@rki.de |
| Corinna | Patzina-Mehling | German Federal Institute of Risk Assessment;<br>corinna.patzina-mehling@bfr.bund.de |
| Natalie | Pauly | German Federal Institute for Risk Assessment;<br>natalie.pauly@bfr.bund.de |
| Ju-Yi | Peng | University of Veterinary Medicine Hanover;<br>ju-yi.peng@tiho-hannover.de |
| Maren | Penning | Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany;<br>maren.penning@fli.de |
| Monique | Petry | TiHo RIZ Research Center for Emerging Infections and Zoonoses;<br>monique.petry@tiho-hannover.de |
| Jana | Petzold | Justus Liebig University Giessen;<br>jana.petzold@vetmed.uni-giessen.de |
| Martin | Pfeffer | Insitute of Animal Hygiene ad Veterinary Public Health;<br>pfeffer@vetmed.uni-leipzig.de |
| Parbati | Phuyal | Goethe University, Frankfurt;<br>phuyalparbati@gmail.com |
| Sandra | Pietzonka | Uniklinikum Düsseldorf Institut für Virologie;<br>sandra.pietzonka@med.uni-duesseldorf.de |
| Aikaterini | Pistiki | Leibniz Institute for Photonic Technologies;<br>aikaterini.pistiki@uni-jena.de |
| Stephan | Pleschka | Institute of Medical Virology, Gießen;<br>stephan.pleschka@viro.med.uni-giessen.de |
| Stefan | Pöhlmann | Deutsches Primatenzentrum GmbH - Infection Biology Unit;<br>spoehlmann@dpz.eu |
| Sven | Poppert | SwissTPH;<br>sven@poppert.eu |
| Julia | Port | Bernhard Nocht Institute for Tropical Medicine;<br>julia.port@bnitm.de |
| Madlin | Potratz | Friedrich-Loeffler-Institut;<br>madlin.potratz@fli.de |

| | | |
|---|---|---|
| Fabian | Pott | Charité - Universitätsmedizin Berlin;<br>fabian.pott@charite.de |
| Joseph | Prescott | Robert Koch-Institut;<br>prescottj@rki.de |
| Christina | Princk | Nds. Landesgesundheitsamt;<br>christina.princk@nlga.niedersachsen.de |
| Marian | Prokes | University of Veterinary Medicine and Pharmacy in Kosice;<br>prokesh@pobox.sk |
| Christoph | Püning | Institute of Food Safety and Food Hygiene, FU Berlin;<br>christoph.puening@fu-berlin.de |
| Konstantin | Pusl | Max von Pettenkofer Institut;<br>pusl@mvp.lmu.de |
| Denise | Rabold | Institut für Mikrobiologie und Tierseuchen;<br>denise.rabold@fu-berlin.de |
| Jessica | Rauch | Bernhard-Nocht-Institute for Tropical Medicine;<br>rauch@bnitm.de |
| Abdul | REHMAN | University of Veterinary and Animal Sciences, Lahore;<br>abdul.rehman@uvas.edu.pk |
| Benjamin | Reichelt | Freie Universität Berlin;<br>benjamin.reichelt@fu-berlin.de |
| Zaida | Renteria | Institut für Parasitologie, Veterinärmedizinische Fakultät, Universität Leipzig;<br>zaida_melina.renteria_solis@vetmed.uni-leipzig.de |
| Caroline | Robé | Institut für Tier- und Umwelthygiene;<br>caroline.robe@fu-berlin.de |
| Katharina | Röpke | none;<br>k-roepke@gmx.de |
| Kerstin | Rosen | Freie Universität Berlin;<br>kerstin.rosen@fu-berlin.de |
| Maria | Rosenthal | Bernhard Nocht Institute for Tropical Medicine;<br>rosenthal@bnitm.de |
| Dennis | Rubbenstroth | Friedrich-Loeffler-Institut;<br>dennis.rubbenstroth@fli.de |
| Elgin | Ruch | Veterinäramt Kreis Gütersloh und Universität Bielefeld, Fakultät der Gesundheitswissenschaften;<br>elgin.ruch@web.de |
| Martin | Runge | LAVES Lebensmittel- und Veterinärinstitut Braunschweitg/Hannover;<br>martin.runge@laves.niedersachsen.de |
| Frank | Salchow | LAVES - Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit;<br>frank.salchow@laves.niedersachsen.de |
| Sharareh | Salehi Hossainy | Robert Koch-Institute;<br>salehishirareh@gmail.com |
| Novra | Sandi | Department of Public Heath and Tropical Medicine, Faculty of Medicine, Public Health and Nursing, University of Gadjah Mada;<br>nsan5881@uni.sydney.edu.au |
| Miriam A. | Sas | altona Diagnostics;<br>miriam.sas@altona-diagnostics.com |
| Mandy | Schäfer | Friedrich-Loeffler-Institut; |

| | | |
|---|---|---|
| | | mandy.schaefer@fli.de |
| Birgit | Schauer | Universitätsmedizin Greifswald;<br>birgit.schauer@uni-greifswald.de |
| Maria | Schellen | Institut für Tierärztliche Nahrungsmittelkunde, Justus-Liebig-Universität Gießen;<br>diana.m.schellen@vetmed.uni-giessen.de |
| Anne-Kathrin | Schink | Institut für Mikrobiologie und Tierseuchen FU-Berlin;<br>anne-kathrin.schink@fu-berlin.de |
| Andreas | Schlattmann | University Hospital Münster;<br>andreas.schlattmann@ukmuenster.de |
| Kore | Schlottau | Friedrich-Loeffler-Institut;<br>kore.schlottau@fli.de |
| Jasmin | Schlotterbeck | Hu Berlin;<br>slotterj@hu-berlin.de |
| Patrick | Schmerer | JLU Giessen;<br>patrick.schmerer@vetmed.uni-giessen.de |
| Jonas | Schmidt-Chanasit | Bernhard Nocht Institut für Tropenmedizin;<br>jonassi@gmx.de |
| Hannah M. | Schmuck | Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig;<br>hannah.schmuck@vetmed.uni-leipzig.de |
| Julia | Schneider | Institute of Virology, Charite;<br>julia.schneider@charite.de |
| Anne | Schnepf | University of Veterinary Medicine Hannover;<br>anne.schnepf@tiho-hannover.de |
| Arne | Schnitt | German Federal Institute for Risk Assessment;<br>arne.schnitt@bfr.bund.de |
| Anissa | Scholtzek | Freie Universität Berlin;<br>a.scholtzek@fu-berlin.de |
| Johannes | Scholz | Federal institute for Risk Assessment, Berlin;<br>johannes.scholz@bfr.bund.de |
| Kathleen | Schön | Tierärztliche Hochschule Hannover, Institut für Parasitologie;<br>kathleen.schoen@tiho-hannover.de |
| André | Schreiber | Institute of Virology, Münster;<br>andre.schreiber@uni-muenster.de |
| Simon | Schroeder | Charité;<br>simon.schroeder@charite.de |
| Angela Regina | Schug | Freie Universität Berlin;<br>a.schug@fu-berlin.de |
| Claudia | Schulz | University of Veterinary Medicine Hannover, Research Center for Emerging Infections and Zoonoses;<br>claudia.schulz@tiho-hannover.de |
| Vanessa | Schulze | Friedrich-Loeffler-Institut, Institut für neue und neuartige Tierseuchenerreger, Greifswald-Insel Riems;<br>vanessa.schulze@fli.de |
| Jan Hendrik | Schwarz | Lehrstuhl für Virologie  Institut für Infektionsmedizin und Zoonosen Tierärztliche Fakultät der LMU;<br>jan.schwarz@micro.vetmed.uni-muenchen.de |
| Ilia | Semmler | Institut für Virologie Charité - Universitätsmedizin Berlin; |

| | | |
|---|---|---|
| | | ilia.semmler@charite.de |
| Torsten | Semmler | Robert Koch Institute, Microbial Genomics;<br>semmlert@rki.de |
| Soroush | Sharbati | Freie Universität Berlin;<br>soroush.sharbati@fu-berlin.de |
| Cornelia | Silaghi | Friedrich-Loeffler-Institut;<br>cornelia.silaghi@fli.de |
| Paul | Siller | Freie Universität Berlin, Institut für Tier- und Umwelthygiene;<br>paul.siller@fu-berlin.de |
| Gisele | Silva Boos | Institute of Veterinary-Pathology, Justus-Liebig-University;<br>gisele.s.boos@vetmed.uni-giessen.de |
| Katharina | Sobotta | Institut für Medizinische Mikrobiologie;<br>katharina.sobotta@med.uni-jena.de |
| Laia | Solano-Gallego | Departament de Medicina i Cirurgia animals, Facultat de Veterinaria, Universitat Autònoma de Barcelona;<br>laia.solano@uab.cat |
| Sebastian | Sprengel | German Research Platform for Zoonoses;<br>sebastian.sprengel@ukmuenster.de |
| Ivonne | Stamm | Vet Med Labor GmbH;<br>ivonne-stamm@idexx.com |
| Klaus | Stark | Robert Koch Institute;<br>starkk@rki.de |
| Mareike | Stellfeld | Friedrich-Loeffler-Institut, Institut für bakterielle Infektionen und Zoonosen;<br>mareike.stellfeld@fli.de |
| Petra | Strakova | Veterinary Research Institute;<br>strakova.p@centrum.cz |
| Ralf | Sudbrak | Global AMR R&D Hub;<br>ralf.sudbrak@dzif.de |
| Beneditta | Suwono | Bundesinstitut für Risikobewertung;<br>beneditta.suwono@bfr.bund.de |
| Vanessa | Szott | Freie Universität Berlin;<br>vanessa.szott@fu-berlin.de |
| Dennis | Tappe | Bernhard-Nocht-Institut;<br>tappe@bnitm.de |
| Wiliam | Tasiame | Institute of Virology, Charite Universitatsmedizin, Campus Mitte;<br>drwilly2002@gmail.com |
| Nicole | Tegtmeyer | Friedrich-Alexander Universität Erlangen-Nürnberg;<br>nicole.tegtmeyer@fau.de |
| Bernd-Alois | Tenhagen | Bundesinstitut für Risikobewertung;<br>bernd-alois.tenhagen@bfr.bund.de |
| Beate | Tenner | Charité - Universitätsmedizin Berlin (Institut für Virologie);<br>beate.tenner@charite.de |
| Dana | Thal | Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit;<br>dana.thal@fli.de |
| Stephanie | Thomas | Department of Biogeography, University of Bayreuth;<br>stephanie.thomas@uni-bayreuth.de |

| | | |
|---|---|---|
| Sumeet Kumar | Tiwari | Robert Koch Institute;<br>sumeet.kumartt@gmail.com |
| Laura | Uelze | Bundesinstitut für Risikobewertung;<br>laura.uelze@bfr.bund.de |
| Markus | Ulrich | Robert Koch - Institut;<br>ulrichm@rki.de |
| Thomas | Vahlenkamp | University of Leipzig;<br>thomas.vahlenkamp@uni-leipzig.de |
| Kira | van Vorst | Freie Universität Berlin, Institut für Mikrobiologie und Tierseuchen;<br>kira.van.vorst@fu-berlin.de |
| Talitha | Veith | Charite Virologie Institut;<br>talitha.veith@charite.de |
| Asisa | Volz | Ludwigs-Maximilans-Universität München;<br>asisa.volz@micro.vetmed.uni-muenchen.de |
| Albrecht | von Brunn | Max von Pettenkofer-Institut, LMU München;<br>vonbrunn@mvp.lmu.de |
| Maren | von Köckritz-Blickwede | University of Veterinary Medicine Hannover;<br>maren.von.koeckritz-blickwede@tiho-hannover.de |
| Martin | Wainaina | German Federal Institute for Risk Assessment;<br>markimwa@gmail.com |
| Saskia | Weber | Friedrich-Loeffler-Institut;<br>saskia.weber@fli.de |
| Sabrina | Weiss | Charité - Universitätsmedizin Berlin;<br>sabrina.weiss@charite.de |
| Laura | Wiesner | Tierärztliche Hochschule - Institut für Physiologische Chemie;<br>laura.wiesner@tiho-hannover.de |
| Dunja | Wilmes | Robert Koch-Institut;<br>wilmesd@rki.de |
| Peter T. | Witkowski | Landesamt für Gesundheit und Soziales;<br>peter.witkowski@lageso.berlin.de |
| Wolfgang | Witte | Robert Koch Institute, Wernigerode Branch;<br>ewwitte@t-online.de |
| Wolfgang | Witte | Rpbert Koch Institute, Wernigerode Branch;<br>cunych@rki.de |
| Silver Anthony | Wolf | Robert Koch Institute;<br>wolfs@rki.de |
| Alexander | Wolff | Bundesinstitut für Risikobewertung;<br>alexander.wolff@bfr.bund.de |
| Andreas | Woschke | RKI;<br>andreas.woschke@charite.de |
| David | Wozniak | Robert Koch-Institute;<br>wozniakd@rki.de |
| De | Xi | Freie Universität Berlin, Institute of Veterinary Biochemistry;<br>xide201110@hotmail.com |
| Janine | Zahlten | LAGeSo Berlin;<br>janine.zahlten@lageso.berlin.de |

| | | |
|---|---|---|
| Silvia | Zemanová | University of Veterinary Medicine and Pharmacy in Košice; slaverein@gmail.com |
| Ina | Zerbin | veterinarian, IVD GmbH; zerbina@posteo.de |

## Personal notes

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