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Welcome Address of the German Research Platform for Zoonoses

Dear colleagues,

We are delighted to welcome you to the National Symposium on Zoonoses Research 2018, which is organized as the first joint meeting of the German Research Platform for Zoonoses and the new Research Network of Zoonotic Diseases that has been established in 2017 as a new project consortium under the umbrella of the Research Platform.
Due to the great demand of our previous symposia and the recent extension of activities, for the first time ever, the Symposium is going to take place over the span of three days.
For this year´s symposium we have again created an agenda that we hope appeals to everyone. This includes keynote presentations by distinguished colleagues as well as selected workshop talks in parallel sessions covering a broad spectrum of topics. In particular, we'd like to highlight the keynotes on Wednesday and Friday as well as a special science communication session on Thursday.
We would like to take this opportunity to thank everyone who has submitted abstracts as well as prepared posters and presentations. All of you are making a significant contribution to the success of this conference.
Once again, our Young Scientists Breakfast will take place on Friday morning. Junior scientists are invited to take advantage of this unique opportunity to discuss topics such as career planning, research experiences and other subjects with experienced colleagues during a casual breakfast session.
The conference is designed as a platform for exchanging up-to-date knowledge, meeting new cooperation partners and intensifying existing partnerships beyond scientific disciplines and geographical boundaries. Let's join forces and bring the One Health ideal to life!

Martin H. Groschup
(Greifswald, Germany)

Stephan Ludwig
(Münster, Germany)

Sebastian C. Semler
(Berlin, Germany)

Christian Drosten
(Berlin, Germany)

Welcome Note of the Federal Government

Grußwort der Bundesregierung zum National Symposium on Zoonoses Research 2018

Sehr geehrte Teilnehmer,

ich freue mich sehr, Sie auch im Namen der an der nationalen Forschungsplattform für Zoonosen beteiligten Bundesministerien, den Ressorts für Bildung und Forschung, für Gesundheit, für Ernährung und Landwirtschaft sowie der Verteidigung, zum diesjährigen 11. Nationalen Symposium für Zoonosenforschung begrüßen zu können. Im Unterschied zu den zweitägigen Symposien der vergangenen Jahre wird sich das Zoonosensymposium 2018 erstmals über einen Zeitraum von drei Tagen erstrecken. Ich werte dies als Zeichen der hohen Relevanz dieser Forschung, der besonderen Attraktivität und des einzigartigen Charakters dieser interdisziplinären Veranstaltung.
Die Bedrohung der Gesundheit von Mensch und Tier durch zoonotische Infektionskrankheiten hat nichts von ihrer Aktualität verloren. Ein begrenzter Ausbruch von Ebola in der Demokratischen Republik Kongo im Mai dieses Jahres ist dabei nur ein Beispiel, welches Eingang in die weltweite Presseberichterstattung gefunden hat. Aber man muss nicht nur auf das Ausland schauen, um die zunehmende Bedeutung der Zoonosen zu erkennen. Tierische Reservoire von multiresistenten humanpathogenen Infektionserregern, das Auftreten eines neuen Bornavirus oder die zunehmende Ausbreitung der Frühsommer-Meningoenzephalitis trotz verfügbarer Impfung zeigen Handlungs- und Forschungsbedarf auch unmittelbar vor unserer Haustür auf.
Mit der Erneuerung der Forschungsvereinbarung zu zwischen Menschen und Tieren übertragbaren Krankheiten vom Januar 2016 hat die Bundesregierung der Bedeutung der Zoonosenforschung Rechnung getragen. Seither beteiligt sich hier auch das Bundesministerium der Verteidigung.
Die Erforschung und Prävention von Zoonosen spielt auch im Bereich der Streitkräfte eine wichtige Rolle. Bei Auslandseinsätzen in den unterschiedlichsten Regionen der Welt mit ihren besonderen Gesundheitsgefahren, unter denen nicht zuletzt die Zoonosen von großer Bedeutung sind, steht der vorbeugende Gesundheitsschutz für die dort eingesetzten Soldatinnen und Soldaten stets im Vordergrund.

Der One- Health-Gedanke wird dabei aktiv gelebt. Vertreterinnen und Vertreter aus Human- und Veterinärmedizin arbeiten interdisziplinär, sowohl im Bereich der Forschung am Institut für Mikrobiologie der Bundeswehr, als auch im Rahmen der Prävention im Inland und vor Ort in den Einsatzländern eng zusammen.
Die Stärkung der Zoonosenforschung steht auch weiterhin im Fokus der Bundesregierung. So ist die Finanzierung der Arbeiten der Forschungsplattform für Zoonosen für weitere vier Jahre durch das Bundesministerium für Bildung und Forschung sichergestellt, welches darüber hinaus seit Mitte 2017 das Forschungsnetz zoonotische Infektionskrankheiten fördert. Weiterhin hat das
Bundesministerium für Gesundheit im Januar dieses Jahres eine Bekanntmachung zu zoonotischen Infektionskrankheiten und Erregern mit speziellen Resistenzen veröffentlicht. Mit einem Budget von vier Millionen Euro werden hierbei Projekte zur genombasierten Surveillance, zur Weiterentwicklung von Sentinel-Systemen sowie zur Erforschung chronisch persistierender zoonotischer Infektionen gefördert. Eine weitere Bekanntmachung, diesmal aus dem Bundesministerium für Ernährung und Landwirtschaft, soll Ende des Jahres folgen.
Das diesjährige Zoonosensymposium wird, wie schon in der Vergangenheit, den geeigneten Rahmen und eine Plattform zum interdisziplinären Gedanken- und Informationsaustausch, zur Diskussion neuer Erkenntnisse und Entwicklungen sowie zur Vernetzung der Expertinnen und Experten auf dem Gebiet der Zoonosenforschung bieten.
In diesem Sinne wünsche ich uns allen eine erfolgreiche Veranstaltung, interessante Vorträge, spannende Diskussionen und vielleicht auch ein wenig Zeit, um Berlin mit seinen zahlreichen Möglichkeiten kennenzulernen und zu genießen.

Wir wünschen damit allen Teilnehmerinnen und Teilnehmern ein spannendes Symposium, interessante Gespräche und zahlreiche neue Ideen für gemeinsame Projekte.

Federal Ministry of Education and Research (BMBF)

Federal Ministry of Health (BMG)

Federal Ministry of Food and Agriculture (BMEL)

Federal Ministry of Defense (BMVg)

Program

Wednesday, October 17, 2018

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

15:00 – 17:00 **Plenary Session I: Keynotes**
(Room Ballsaal)
Language: English / German
Chair: Stephan Ludwig

15:00 **Opening Remarks:**
Joint meeting *of the German Research Platform for Zoonoses and the Research Network of Zoonotic Diseases*
Stephan Ludwig (University of Münster) & Christian Drosten (Charité - Universitätsmedizin Berlin)

Welcome Note of the Federal Government
Michael Engels (Federal Ministry of Defense)

15:30 **Keynote 1:**
Zoonotic risk and unmet needs in the One Health era
Alexandra Mailles, Saint-Maurice, France

16:15 **Keynote 2:**
NGS, big data and molecular epidemiology
Rene S. Hendriksen, Lyngby, Denmark)

17:00 *Coffee Break*

17:20 – 18:50 Session 1: Pathogenesis and Modelling of Zoonotic Diseases
(Room Ballsaal)
Language: English
Chairs: *Martin Beer and Rainer Ulrich*

17:20 **Function of serine protease HtrA in the lifecycle of the foodborne pathogen *Campylobacter jejuni***
D. Simson, **S. Backert**, M. Boehm

17:35 **O156:H25/O182:H25 STEC of human and bovine origin share remarkable genomic similarity**
C. Menge, S.A. Barth, M. Fischer, A. Fruth, C. Berens, L. Geue

17:50 **Establishment of a loss-of-function screening platform to identify MERS-Coronavirus host factor dependencies**
F. Weege, D. Muth, A. Karlas, C. Drosten, T. F. Meyer

18:05 **Identification of intestinal luminal metabolites mediating colonization resistance against Campylobacteriosis in murine infection models**
S. Bereswill, U. Escher, K. Stingl, M. M. Heimesaat

18:20 **Immunogenicity and protective capacity of recombinant Modified Vaccinia Ankara Virus against Zika Virus**
J. H.Schwarz, D. Forster, **A. Volz**

18:35 **Analysis of proteolytic activation of the spike protein of MERS coronavirus**
H. Kleine-Weber, M. Tarek, M.Hoffmann, S. Pöhlmann

18:50 *Poster Viewing Session*

Thursday, October 18, 2018

08:30 Registration continued

09:00 – 10:30 Session 2: Risk Assessment and Epidemiology
(Room Ballsaal)
Language: English
Chairs: *Ard Nijhof & Stephanie Thomas*

09:00 **Can we predict the spatial origin of hantavirus infections using sequences derived from a generic screening PCR?**
S. Weiss, B. Auste, D. H Kruger, J. Hofmann

09:15 **Molecular characterization and drug susceptibility of *Mycobacterium tuberculosis* from Eastern Sudan**
Y. A. Shuaib, M. Merker, E. A. G. Khalil, U. Schaible, L. Wieler, M. A. Bakheit, S. E.-T. Mohamed-Noor, M. A. Abdalla, E. Richter, K. Kranzer, S. Niemann

09:30 **Chikungunya in Germany: Where and when could it happen?**
N. Tjaden, F. Englmeier, K. Nagels, S. Thomas, **C. Beierkuhnlein**

09:45 **Evaluation of the zoonotic potential of H18N11 virus variant tested in the ferret model**
M. Gorka, D. Hoffmann, K.Ciminski, M. Schwemmle, M. Beer

10:00 **Multidrug-resistant zoonotic bacteria in companion animals from North-West Germany**
U. Kaspar, K. von Lützau, A. von Lützau, A. Schlattmann, G. Peters, U. Rösler, R. Köck, K. Becker

10:15 **Zoonoses in Exotic Pets - Epidemiologic Survey on the Variegated Squirrel Bornavirus 1 in Captive Squirrels (Family Sciuridae) in Germany**
V. Allendorf, K. Schlottau, V. Schulze, F. J. Conraths, R.G. Ulrich, M. Beer, T. Homeier-Bachmann

09:00 – 10:30 Session 3: Innate and Adaptive Immune Response
(Room Zehlendorf)
Language: English
Chairs: *Asisa Volz & Veronika von Messling*

09:00 **Interferon response is essential in containing human pathogenic Bourbon virus, a tick-borne Orthomyxovirus**
J. Fuchs, T. Straub, M. Seidl, G. Kochs

09:15 **GlykoViroLectinTools: novel mosquito and sheep C-type lectin receptor (CLR)-Fc fusion protein libraries to screen for CLR/pathogen interactions**
D. Lindenwald, J. Monteiro, K. Schoen, J. Glanz, A. Sternberg, S. Rautenschlein, M. Buettner, G. Alber, K. Jung, S. Becker, B. Lepenies

09:30 **Functional characterization of the interferon-induced antiviral factor tetherin of fruit bats**
M. Hoffmann, I. Nehlmeier, C. Brinkmann, V. Krähling, L. Behner, A.-S. Moldenhauer, N. Krüger, J. Nehls, M. Schindler, A. Maisner, S. Becker, S. Pöhlmann

09:45 **MERS-Coronavirus strain with a fusion of two accessory genes isolated from a human patient**
D. Niemeyer, K. Mösbauer, E. M. Klein, D. Muth, V. Corman, M. A. Müller, Z. A. Memish, C. Drosten

10:00 **Regions-specific regulation of type I interferon in the central nervous system protects from tick-borne encephalitis virus infection**
S. Schreier, L. Zegenhagen, C. Kurhade, A. K. Överby, **A. Kröger**

10:15 **Murine fecal microbiota transplantation lowers intestinal *Campylobacter jejuni* loads and pro-inflammatory immune responses in secondary abiotic mice**
M. M. Heimesaat, U. Escher, S. Bereswill

09:00 – 10:30 Session 4: Antimicrobial use and resistance (Room Steglitz)
Language: English
Chairs: *Denise Rabold & Birgit Walter*

09:00 **Wastewater from a pig slaughterhouse as a reservoir for clinically relevant antibiotic-resistant pathogens and their dissemination into surface water**
M. Savin, C. Heinemann, S. Dohlen, G. Bierbaum, M. Parcina, J. Kreyenschmidt

09:15 **Development of antibiotic resistance in fattening poultry**
C. Kollas, M. Grobbel, A. Käsbohrer, B. A. Tenhagen, A. A. Weiser

09:30 **Characterization of a genetically encoded Tetracycline resistance determinant in *Chlamydia suis***
M. Peisker, C. Schnee, C. Berens

09:45 **Identification of new mcr-3 colistin resistance gene variants in *Aeromonas* spp. of animal origin from Germany**
I. Eichhorn, C. Feudi, A.T. Feßler, G. Brenner-Michael, Y. Wang, H. Kaspar, A. Lübke-Becker, J. Shen, S. Schwarz

10:00 **Analysis of blaCTX-M-1-carrying plasmids from *Escherichia coli* isolates of diseased food-producing animals collected in the GERM-VET resistance monitoring program 2008-2015**
G. B. Michael, **A.-K. Schink**, K. Kadlec, H. Kaspar, S. Schwarz

10:15 **High zinc oxide diets: effects on porcine intestinal *Escherichia coli* populations**
V.C. Johanns, T. Semmler, L. Epping, B. Walther, L.H. Wieler

10:30 Coffee Break and Poster Viewing

11:00 – 12:30 Session 5: Selected Plenary Talks of the Research Network of Zoonotic Infectious Diseases
(Room Ballsaal)
Language: English
Chairs: *Christian Drosten & Martin Blume*

11:00 **Promising options to combat campylobacteriosis - lessons learned from peroral gut microbiota transplantation and application of defined molecules in murine infection models**
M. M. Heimesaat

11:30 **Antibiotic Stewardship in Veterinary and Human Medicine**
R. Köck

12:00 **An unexpected development – classical bornavirus BoDV-1 as lethal zoonotic pathogenes**
M. Beer

12:30 Lunch & Poster Viewing

14:30 – 16:00 Session 6: New and Re-Emerging Zoonotic Diseases
(Room Ballsaal)
Language: English
Chairs: *Andrea Rasche & Martin H.- Groschup*

14:30 **Experimental risk assessment for chikungunya virus transmission in Europe**
A.Heitmann, S. Jansen, **R. Lühken**, M. Helms, B. Pluskota, N. Becker, C. Kuhn, J. Schmidt-Chanasit, E. Tannich

14:45 **Novel sandfly-associated phlebovirus with evidence of neutralizing antibodies in humans, Kenya**
D. Tchouassi, **M. Marklewitz**, E. Chepkorir, F. Zirkel, S. Agha, C. Tigoi, E. Koskei, C. Drosten, C. Borgemeister, B. Torto, S. Junglen, R. Sang

15:00 **CRISPR-Forward screening approach to identify host cell factors required for bat influenza A-like virus (bat IAV) cell entry**
T. Thamamongood, U.Karakus, K. Ciminski, W. Ran, G. Zimmer, M. Beer, A. Garcia-Sastre, S. Stertz, M. Schwemmle

15:15 **Cowpox Virus - Host Interactions: Identification and Confirmation of Virulence Factors**
S. Weber, A. Franke, D. Hoffmann, A. Tamošiūnaitė, T. Schippers, K. Osterrieder, M.Beer

15:30 **Human pathogenic *Leptospira* in small mammals**
S. Fischer, K. Jeske, A. Mayer-Scholl, F. Ruiz-Fons, C. Imholt, E. Heuser, J.P. Teifke, D. Reil, J. Jacob, K. Nöckler, L. Bluhm, A. Breitenstein, W. Fritzsche, R.G. Ulrich

15:45 **Neutralizing monoclonal antibodies and alpaca derived single domain antibody fragments against Rift Valley fever virus**
B. Gutjahr, M. Eiden, S. Jäckel, S. Reiche, M. H. Groschup

14:30 – 16:00 Session 7: Public Health
(Room Steglitz)
Language: English
Chairs: Sascha Al-Dahouk & Uwe Rösler

14:30 **Activity and prevalence of pathogens in *Ixodes inopinatus* and *Ixodes ricinus* in Southeastern Germany**
L. Chitimia-Dobler, S. Wölfel, G. Lemhöfer, Bestehorn, G. Dobler

14:45 **Areas with high hazard potential for autochthonous transmission of *Aedes albopictus* associated arboviruses in Germany**
S. Thomas, N. Tjaden, C. Frank, A. Jaeschke, L. Zipfel, C. Wagner-Wiening, M. Faber, C.Beierkuhnlein, K. Stark

15:00 **Reduction of *Escherichia coli* cell numbers by colicins**

A. Rößner, J. Andrack, G.Gölz, T. Seidler, T. Forbrig, T. Alter, S. Orquera

15:15 **One Health Interventions of Vétérinaires sans Frontières Germany in the current anthrax outbreak in South Omo Zone, Ethiopia**
A. Braus, A. R. Schug, M. V. Larrateguy, A. Asrat, A. Adamu, G. Regassa

15:30 **Genotypic and phenotypic characterization of *Listeria monocytogenes* originating from food production environments**
A. Roedel, **S. Vincze**, M. Noll, S. Kleta, S. Al Dahouk, R. Dieckmann

15:45 **Health economic assessment of a possible chikungunya transmission in Germany**
F. Englmeier, N. B. Tjaden, S. M. Thomas, C. Beierkuhnlein, K. H. Nagels

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16:00 Coffee break and Poster Viewing

Impulse Session "Science Communication"
(Room Ballsaal)
Language: German
Chair: *Stephan Ludwig*

16:30 **Talk 1: Andrea Maisner (Philipps Universität Marburg)**

16:50 **Talk 2: Kai Kupferschmidt**

17:10 **Talk 3: Volker Stollorz (Science Media Center Germany)**

17:30 – 19:30 **General Assembly German Research Platform for Zoonoses (Room: Ballsaal)**
Language: German
Chairs*: Stephan Ludwig, Martin H. Groschup, Sebastian C. Semler*

19:00 *Poster Viewing Session + Casual Reception*

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

Friday, October 19, 2018

07:30 – 09:30 Young Scientist Breakfast

09:30 – 10:30 Session 8: Pathogen-Cell Interaction
(Room Zehlendorf)
Language: English
Chairs: Imke Steffen & Anja Lührmann

09:30 **Bacterial nucleases as growth factor during bacterial co-infections**
N. de Buhr, B. J. Pfeiffer, S. Akhdar, C. Schwennen, K.-H. Waldmann, P. Valentin-Weigand, I. Hennig-Pauka, M. von Köckritz-Blickwede

09:45 **Hypoxia-induced citrate limitation results in upregulation of *C. burnetii* persistence genes**
I. Hayek, F. Fischer, J. Schulze-Luehrmann, K. Dettmer-Wilde, R. Lang, P. Oefner, S. Wirtz, J. Jantsch, A. Lührmann

10:00 **Generation and characterization of synthetically-derived bat mumps virus**
N. Krüger, C. Sauder, S. Hüttl, K. Voigt, G. Herrler, K. Hardes, T. Steinmetzer, C. Örvell, J. F. Drexler, M. Müller, C. Drosten, S. Rubin, M. Hoffmann

10:15 **A cell model to investigate ENaC-dependent sodium absorption and tight junction alterations in campylobacteriosis**
P. Nattramilarasu, F. Lobo de Sá, H. Tröger, J.D. Schulzke, R. Bücker

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09:30 – 10:45 Session 9: Novel Methods, Diagnostics and NGS
(Room Steglitz)
Language: English
Chairs: Caroline Herr & Martin H. Groschup

09:30 **Monitoring wildlife and their pathogens using flies**
S. Calvignac-Spencer, F. H. Leendertz

09:45 **Successful oral vaccination against HPAIV H5N1 with novel bat influenza A virus chimeras**
J. Schön, W. Ran, D. Hoffmann, M. Gorka, M. Juozapaitis, M. Schwemmle, M. Beer

10:00 **Exploring "big sequence data": De-novo detection of viral pathogens by a dynamic database approach**
L. Forth, D. Höper, A. Hülsewig, R. Lembcke, C. Peißert, P. Holenya, M. Eckey, U. Reimer, K. Noack, M. Beer, **A. Pohlmann**

10:15 **Persistent virus infection in mosquito derived cells**
E. Schnettler, K. Franzke, M. Leggewie, S. Vatipally, E. Tannich, S.C. Becker

10:30 **Gene expression and signaling pathways in the human colon mucosa during *Campylobacter jejuni* enteritis**
R. Bücker, F. Lobo de Sá, M. Kerick, M.R. Schweiger, J.-D. Schulzke

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09:30 – 11:00 Session 10: Epidemiology and Ecology of Zoonotic Infections
(Room Ballsaal)
Language: English
Chairs: Helge Kampen & Sandra Junglen

09:30 **Epidemiology of tick-borne encephalitis in Germany in 2017**
G. Dobler

09:45 **Tick-borne encephalitis virus goes into the mountains**
L. Lemhöfer, M. Bestehorn, L. Chitimia-Dobler, G. Dobler

10:00 **Field work of the AECO project - Vector biology of *Aedes albopictus* and eco-bio-social drivers for effective vector prevention & control in cooler ecoregions**
R. Müller, I. Kramer, P. Phuyal, U. Kuch, D. A. Groneberg, M. Dhimal

10:15 **Environmental occurrence of *Campylobacter spp.* in broiler farms and surroundings**
B. Reichelt, V. Szott, K. Daehre, U. Roesler

10:30 **Clustering of hantavirus disease cases in a company in Lower Saxony, Germany, in December 2017, caused by intense bank vole infestation**
K.M. Schlinkmann, C. Princk, S. Drewes, RG. Ulrich, M. Saathoff, J. Freise, J. Dreesman

10:45 **Ticks and tick-borne pathogens in Sudan**
Y.A.Shuaib, MA.Abdalla, SE-T. Mohmed-Noor, AMA. Wd Elhag, YAB Ismael, G. Lemhöfer, S. Poppert, S. Schaper, G. Dobler, DK. Bakkes, **L. Chitimia-Dobler**

11:00 Coffee Break & Poster Viewing

11:30 – 12:15 Plenary Session II: Keynote (Room Ballsaal)
Language: English
Chair: *Martin H. Groschup*

11:30 Keynote 3:
Leptospirose-Ausbruch bei Erdbeerpflückern 2014
Leonard Hamschmidt, Oldenburg, Germany

12:15 Lunch & Poster Viewing

13:15– 14:30 Plenary Session II (continued): Keynote (Room Ballsaal)
Language: English
Chair: *Martin H. Groschup*

13:15 **Keynote 4:**
Preparedness for zoonoses and emerging arboviral infections
Xavier de Lamballerie, Marseille, France

14:30 **Poster Awards & Farewell**

General Information

Date and Venue
October 17-19, 2018

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

How to get there

The venue is accessible within 15 minutes from Berlin Tegel Airport and within 25 minutes from Berlin Schönefeld Airport. You can travel by car or by public transportation. A subway (U-Bahn) and a city railroad station (S-Bahn) are located in front of the hotel. The hotel features an indoor parking garage and offers special parking rates to hotel guests.

Public transportation:
From Berlin Tegel Airport: Take Bus 109 to "Zoologischer Garten". From there, take Subway U9 to "Rathaus Steglitz".

From Berlin Schönefeld Airport: Take Train S45 to "Schöneberg". From there, take Train S1 (direction "Wannsee") to "Rathaus Steglitz".

From Berlin Central Station: Take Train S5, 7 or 75 to "Zoologischer Garten". From there, take Subway U9 to "Rathaus Steglitz" or take Train RB14, S5, 7 or 75 from Berlin Ceentral station to "Friedrichstraße". From there take Train S1 to "Rathaus Steglitz".

Conference Languages
The official conference languages are English and German.

Steering Committee
Martin H. Groschup (Greifswald - Insel Riems)
Stephan Ludwig (Münster)
Sebastian C. Semler (Berlin)

Organization
Office of the German Research Platform for Zoonoses

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Stephan Ludwig
Friederike Jansen
Sebastian Sprengel

Greifswald - Insel Riems:
Martin H. Groschup
Nils Kley

Berlin:
Sebastian C. Semler
Kerstin Splett

With kind support by
Juliane Gehrke (Berlin)
Julia Hartlaub (Greifswald)
Björn Kaeß (Greifswald)
Patrick Wysocki (Greifswald)

Research Network of Zoonotic Infectious Diseases
Christian Drosten
Ilia Semmler

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Veronika von Messling, Langen, Germany
Birgit Walther, Berlin, Germany

Poster Award Committee
The poster awards are selected by the members of the Internal Advisory Board of the German Research Platform for Zoonoses.

Keynote Speakers
Rene S. Hendriksen, Lyngby, Denmark

Xavier de Lamballerie, Marseille, France
Leonard Hamschmidt, Oldenburg, Germany
Alexandra Mailles, Saint-Maurice , France

Session "Science Communication"
Kai Kupferschmidt, Berlin, Germany
Andrea Maisner, Marburg, Germany
Volker Stollorz, Cologne, Germany

Young Scientists Breakfast
The Young Scientists Breakfast is going to take place at the "Pavillon" room of the hotel on Friday, October 19, at 7:30 am.
The attending senior scientists are:

Stefanie Becker, Hannover, Germany
Stefan Bereswill, Berlin, Germany
Donata Hoffmann, Isle of Riems, Germany
Uwe Rösler, Berlin, Germany
Imke Steffen, Hannover, Germany

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
The National Symposium on Zoonoses Research 2018 is registered for **3 CME points of category A on the first and third** as well as **6 CME points of category A for the second day** by the Berlin Chamber of Physicians (Ärztekammer Berlin). Please note that you will need one barcode label per day for the confirmation of participation.

Continuous Veterinary Education
The National Symposium on Zoonoses Research 2018 is registered for **12 hours (ATF-Stunden)** by the Federal Chamber of Veterinarians (Bundestierärztekammer). You will receive your certificate during the lunch break on the second day of the symposium.

Poster Presentations
Posters will be presented during all three days of the conference. 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.

Oral Presentations
Oral presentations should be handed over on a common data carrier at the registration desk starting Wednesday, October 17, between 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.

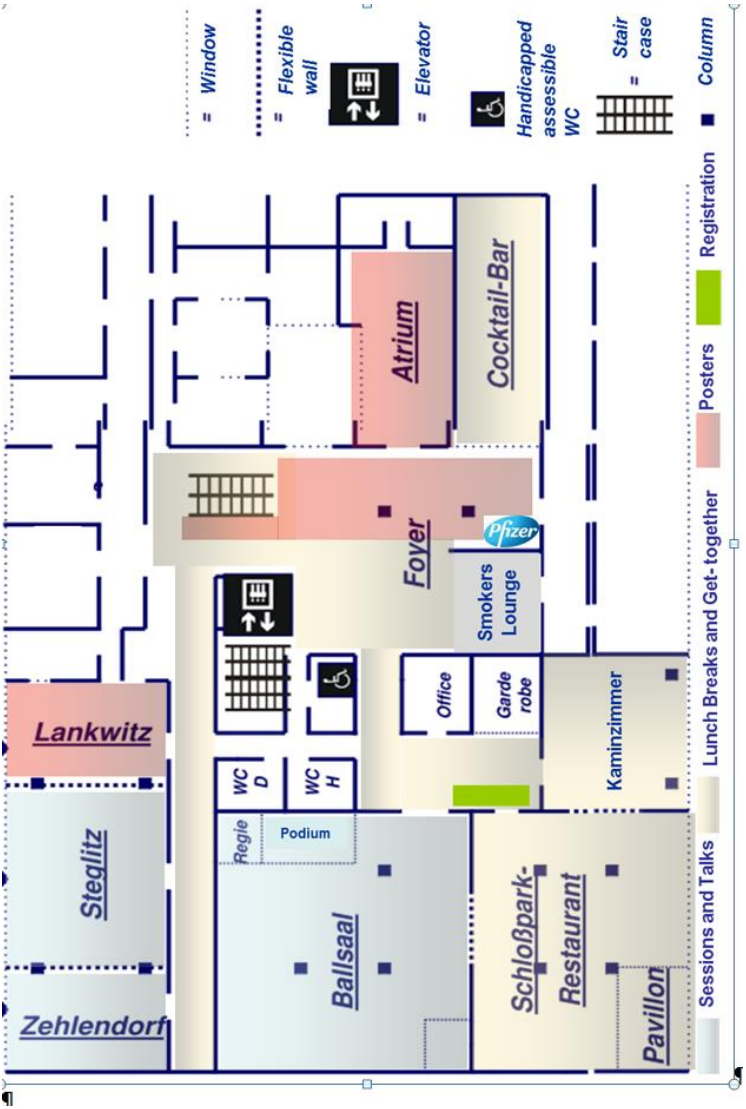
Funding
The National Symposium on Zoonoses Research is funded by the Federal Ministry of Education and Research.

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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Zehlendorf
Ballsaal
Regie
Podium
WC D
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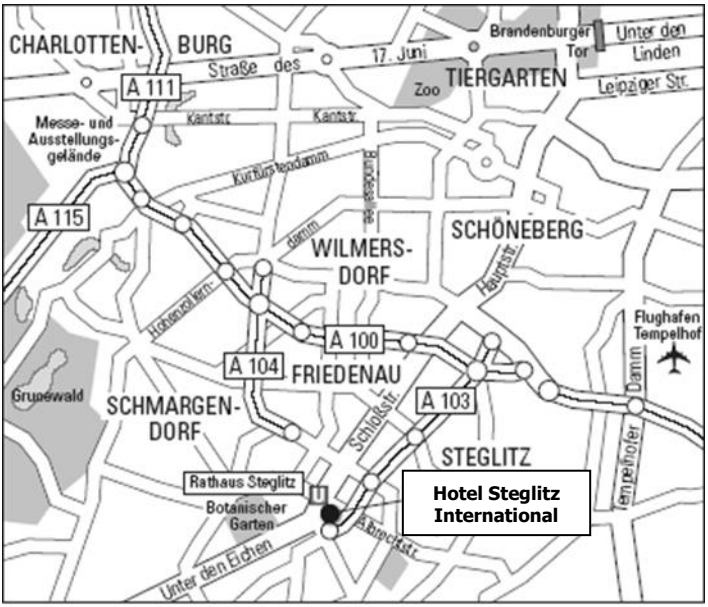
Foyer
Atrium
Cocktail-Bar
Pfizer
Smokers Lounge
Office
Garde robe
Kaminzimmer
Schloßpark-Restaurant
Pavillon

Site Plan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Kantstr.
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SCHÖNEBERG
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Flughafen Tempelhof
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A 104
FRIEDENAU
A 103
Grunewald
SCHMARGEN-DORF
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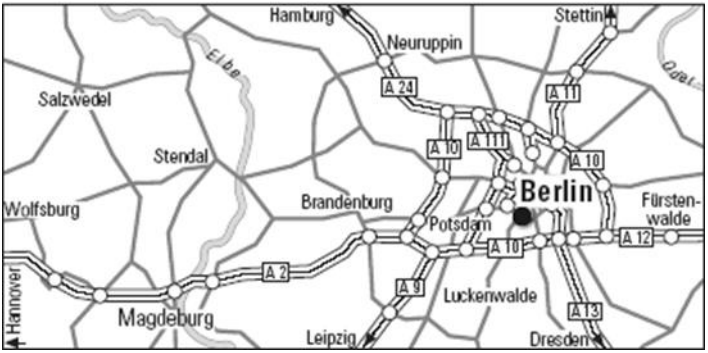
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About the German Research Platform for Zoonoses

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 up to 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 Sessions

October 17, 2018
15:00 – 17:00
Room: Ballsaal
Chair: Stephan Ludwig

and

October 19, 2018
11:30 – 14:15
Room: Ballsaal
Chair: Martin H. Groschup

Plenary Session I: Keynotes

October 17, 2018
15:00 – 17:00

Room Ballsaal
Chair: Stephan Ludwig

Zoonotic risk and unmet needs in the One Health era

A. Mailles[1]

[1]French Public Health Agency, Saint-Maurice, France

Keywords: environmental change, virus infections, wildlife

Most emerging infectious diseases are zoonotic diseases, directly transmitted by animals, or acquired through vector-borne or food-borne transmission. Anticipating the response and implementing appropriate measures in both humans and animals against zoonosis requires understanding the mechanisms supporting their emergence and spread, more precisely the interactions between humans, animal reservoirs, vectors and the environment. A significant number of these mechanisms are human-driven: human activities damaging biotopes occupied by domestic or wild animals; lifestyle increasing the risk of severe infections; climate change responsible for the spread of vectors... They result in increased interactions between humans/domestic animals and wildlife, therefore increasing the risk of introduction of infectious agents in a naive species. The measures to prevent such events are better management of domestic species and their contacts with wildlife, educating the public about infectious diseases risks and the benefits of preserving animal species, better management of the environment. The keystone of the fight against zoonosis is a multidisciplinary collaboration between physicians, veterinarians, environment scientists, but also decision makers and opinion leaders who influence the direction of modern society and are able to transmit the relevant information to the public. Therefore One Health should focus on research, management, preservation and education.

"NGS, big data and molecular epidemiology"

R.S. Hendriksen[1], X. Deng[2], F. M. Aarestrup[1]

[1]Technical University of Denmark, National Food Institute, WHO Collaborating Center for Antimicrobial Resistance in Foodborne Pathogens and Genomics and European Union Reference Laboratory for Antimicrobial Resistance, DK-2800, Kgs. Lyngby, Denmark; [2]Center for Food Safety and Department of Food Science and Technology, University of Georgia, Griffin, Georgia, United States of America

Keywords: Next Generation Sequencing, Genomic Epidemiology, Global Surveillance

In 1995, the first two complete bacterial genome sequences were published and Next Generation Sequencing (NGS) has since changed the landscape of microbiology.
The recent advances in sequencing technologies and bioinformatics tools combined with the steady decline of the sequencing cost have made NGS a viable and advanced solution for epidemiologic investigation and surveillance of bacterial pathogens.
Several pipelines or tools have been developed to enable the inference of traditional subtyping methods, using assembled genomes or raw reads as input. The web-based tools of the Center for Genomic Epidemiology are noteworthy and include, among others, tools to infer MLST types, serotypes of foodborne pathogens, virulence and antimicrobial resistance predictions and latest a tool for metagenomics.
The term "genomic epidemiology" has been increasingly used to describe the practice of utilizing NGS data to access and analyze sequence features of epidemiologic importance. The use of NGS, including metagenomics could likely be expanded to potentially establish a global surveillance of the healthy population around the world using urban sewage to detect and analyse the presence of all pathogens and associated antimicrobial resistance genes, which are essential in disease control and prevention strategies.
The expansion using NGS also brings some challenges, which needs to be addressed in the future such as data sharing, curation of databases, missing standards and QC thresholds.

Plenary Session II: Keynotes

October 19, 2018
11:30 – 14:15

Room Ballsaal
Chair: Martin H. Groschup

Leptospirose-Ausbruch bei Erdbeerpflückern 2014

L. Hamschmidt[1], J. Dreesman[2], J. Freise[3]

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Keywords: Leptospirose-Ausbruch, interdisziplinäre Zusammenarbeit

Hintergrund: Im Sommer 2014 wurden in zwei benachbarten niedersächsischen Landkreisen 45 Fälle von Leptospirose bei Erdbeerarbeitern registriert.
Methoden: Untersuchung des Ausbruchs durch Veterinär- und Gesundheitsbehörden von Kommune, Land und Bund:
-Sammlung von epidemiologischer Daten
-Laboruntersuchung der erkrankten Fälle
-Fang von Mäusen
-Laboruntersuchung der gefangenen Mäuse
-Erfassung von Wetterdaten
-Information an Erzeuger, Feldarbeiter und Ärzte
Ergebnisse: Von 45 registrierten Leptospirose-Fällen (27 männlich, Altersmedian 22 Jahre) wurden 47% stationär behandelt. 15 Fälle wurden serologisch oder molekularbiologisch bestätigt. Als vermutlich verursachender Erreger wurde *L. kirschneri* serovar Grippotyphosa identifiziert. Während der errechneten Infektionszeit herrschte sehr warmes Wetter mit hohen Niederschlägen.
Schlussfolgerungen: Die Untersuchungen bestätigen den Infektionsweg von den Mäusen zu den Feldarbeitern. Im Jahr 2015 und 2016 wurde eine Informationskampagne für Ärzte, Erdbeererzeuger und Feldarbeiter durchgeführt um auf Prävention und frühzeitige Erkennung und Behandlung von Erkrankungen hinzuwirken.

Impulse Session "Science Communication"

October 18, 2018
16:30 – 17:30

Room: Ballsaal
Chairs: Martin H. Groschup, Stephan Ludwig and Sebastian C. Semler

"Marphili-Simulation": A student teaching project including exercises in risk communication

A. Maisner

Institute of Virology, Philipps University, Marburg, Germany

Keywords: simulation project, virus outbreak

The Marphili simulation project outlines a fictional but realistic scenario in which a new virus (Marphili virus) arrives in Germany via returning travelers. The students are requested to act as virologists to clarify the following questions: Who is infected with the new virus? Has the virus already spread further? And how dangerous is the infection for the population? In the lab part, the students first analyze blood samples from patients and potential contact persons. After evaluation of the virus diagnostic results, a press conference is scheduled in which the students take on different roles to inform the press and the public about the current outbreak scenario. Here, it is important to respond objectively to critical and provocative public issues.
Fictional virus outbreaks are also regularly scenarios in movies, with laypersons generally unable to distinguish between fiction and reality. Thus, scientists and (bio-) medical students are often asked for clarifications. They should therefore be able to assess the dramatized facts and risks in films. To practice this during the Marphili simulation, a movie about the topic is shown and subjected to a "reality check". Students should then objectively state what is scientifically correct in the film, what is dramatically exaggerated, and what simply fiction is.

The keynote lecture will be given in German.

Session 1: Pathogenesis and Modelling of Zoonotic Diseases

October 17, 2018
17:20 – 18:50

Room Ballsaal
Chairs: Martin Beer & Rainer Ulrich

Function of serine protease HtrA in the lifecycle of the foodborne pathogen *Campylobacter jejuni*

M. Böhm[1], A. Harrer[1], N. Tegtmeyer[1], S. Backert[1]

[1]Friedrich Alexander University Erlangen/Nuremberg, Department of Biology, Institute for Microbiology, Erlangen, Germany

Keywords: Campylobacter jejuni, HtrA, paracellular transmigration

Background and objectives: *Campylobacter jejuni* is a major foodborne zoonotic pathogen, responsible for a large proportion of bacterial gastroenteritis cases as well as Guillian-Barré and Miller-Fisher syndromes. During infection of humans, tissue damage is mainly caused by bacteria invading epithelial cells and traversing the intestinal barrier. *C. jejuni* is able to enter the lamina propria and the bloodstream, and may move into other organs such as spleen, liver or mesenteric lymph nodes. However, the involved molecular mechanisms are not fully understood.
Materials and methods: Various HtrA mutants were used for transmigration and gentamicin protection assays to determine the adherence, invasion and transmigration rates in polarized human MKN28 and Caco-2 cells. We also performed *in vitro* and *in vivo* cleavage assays to identify HtrA substrates in the host.
Results: *C. jejuni* can transmigrate effectively across polarized intestinal epithelial cells mainly by the paracellular route using the serine protease HtrA. However, it appears that HtrA has a dual function, as it also acts as a chaperone, interacting with denatured or misfolded periplasmic proteins during stress conditions. We report that HtrA can be transported into the extracellular space and cleaves cell-to-cell junction factors such as E-cadherin and occludin, disrupting the epithelial barrier and enabling paracellular transmigration of the bacteria.
Conclusion: The secretion of HtrA is a newly discovered virulence strategy utilized by *C. jejuni* and other pathogens. Thus, secreted HtrA proteases represent highly attractive targets for anti-bacterial treatment and may provide a suitable candidate for vaccine development.

O156:H25/O182:H25 STEC of human and bovine origin share remarkable genomic similarity

C. Menge[1], S.A. Barth[1], M. Fischer[1], A. Fruth[2], C. Berens[1], L. Geue[1]

[1]Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany; [2]Robert-Koch-Institut, Wernigerode, Germany

Keywords: STEC, human, cattle

Escherichia coli realizes a remarkable genome dynamic and flexibility but some strains may be more conserved than others. In cattle, the natural habitat of zoonotic Shiga toxin-producing *E. coli* (STEC), we identified a O156/O182:H25 STEC subpopulation which persist over months and compared these with O156 and O182 STEC strains from diseased humans (diarrhea, HUS) or healthy individuals. Overall, 43 bovine, 11 human, and 3 STEC strains from other animals were characterized. All strains expressing an H25 antigen, irrespective of the O antigen or the host, clustered in a single MLST group (ST300, ST688, ST5343, ST801) that did not differ in more than three single allele polymorphisms. In contrast, strains with other H antigens were separated by at least six different alleles from the H25 positive strains. Based on the analysis of 124 virulence associated genes (VAG), 18/22 O182:H25 strains from humans or cattle showed a strong relatedness with only 7/124 tested VAG being different and, in one case, even clustered in an identical VAG pattern. Our data shows that human and bovine O156:H25 and O182:H25 STEC are closely related or undistinguishable even though the bovine and human strains were isolated 1997–1999 and 2012–2016, respectively. Certain STEC strains stand out in that they seem to be genetically stable over time and in different hosts, optimized for persistent colonization in cattle yet still posing a significant human health risk.

Establishment of a loss-of-function screening platform to identify MERS-Coronavirus host factor dependencies

F. Weege[1], D. Muth[2], A. Karlas[1], C. Drosten[2], T. F. Meyer[1]

[1]Max Planck Institute for Infection Biology, Dept. of Molecular Biology, Berlin, Germany; [2] Institute of Virology, Charité University Medicine, Berlin, Germany

Keywords: MERS-CoV, Screen, CRISPR/Cas9

Background and objective: Zoonotic viruses undergoing onward transmission in humans bear a high pandemic potential. Profound understanding of virus-host interactions is essential for risk assessment, but often remains elusive. Using MERS-CoV as a paradigmatic pre-pandemic virus, we aim to establish a CPE-based screening platform to determine host factor dependencies.
Materials/methods: Infection of human cell lines (native or transduced with sgNAs/libraries) with rMERS-CoV and viability/replication assays; gDNA extraction from resistant cells, amplification of lentiviral integration sites and NGS to quantify sgRNA representation.
Results: We have identified CaCo2 as a suitable cell line showing 100% infection-induced CPE and being amenable to CRISPR/Cas9 genome editing, since a receptor KO rendered cells resistant.
Conclusion: Establishment of a CPE-based CRISPR/Cas9 screening platform allowing for timely screening and elucidation of host factor requirements of emerging CoV is feasible.

Identification of intestinal luminal metabolites mediating colonization resistance against Campylobacteriosis in murine infection models

S. Bereswill[1], U. Escher[1], K. Stingl[2], M. M. Heimesaat[1]

[1]Institute of Microbiology & Infection Immunology, Charité, Berlin, Germany;
[2]National Reference Laboratory for Campylobacter, German Federal Institute for Risk Assessment (BfR), Berlin, Germany
[1,2] PAC-Campylobacter consortium

Keywords: Campylobacteriosis, colonization resistance, metabolomes

Background and objectives: Detailed knowledge about intestinal luminal metabolites providing colonization resistance (CR) of mice against *C. jejuni* (Cj) is well suited to develop strategies directed against *Campylobacter* colonization and infection in farm animals and humans, respectively. In order to identify metabolites combating Cj in the gut we investigated the metabolomes of mice with and without CR.
Materials and methods: The intestinal metabolomes were analysed by Metabolomics Discoveries (Potsdam, Germany) in mice with and without CR. Briefly, colonization resistance is abrogated in mice treated with antibiotics such as ampicillin, ciprofloxacin, vancomycin, metronidazole or imipenem (single and in combination), in conventional infant mice, and in mice harbouring a human microbiota. Conventional adult mice served as controls with CR.
Results: Analysis of the intestinal metabolomes in mice with and without CR against Cj indicated that phenolic compounds might be involved in mediating CR against *Campylobacter*.
Conclusion: Gut metabolites conferring CR will be used for prevention of Cj colonization in poultry as well as for treatment capacities in murine infection models within the PAC-Campylobacter consortium. Murine infection models will aid to validate preventive or therapeutic measures for the final transfer to the pharmaceutical or product level in humans or farm animals, respectively.

Immunogenicity and protective capacity of recombinant Modified Vaccinia Ankara Virus against Zika Virus

D. Forster[1], J. H. Schwarz[1], A. Volz[1]

[1]Institute for Infectious Diseases and Zoonoses, LMU München, Germany

Keywords: Zika virus, vaccination, MVA

Background and objectives: The recent outbreak of Zika Virus (ZIKV) has threatened global populations. ZIKV infection can trigger Guillain-Barré syndrome, and prenatal infection results in dramatically increased number of microcephaly cases in fetuses and newborn children.
There are no vaccines or antiviral drugs available against ZIKV infection. Thus the development of a novel approach for innovative and rational design of vaccines is urgently needed.
Materials and methods:
Modified Vaccinia virus Ankara (MVA), a highly attenuated strain of vaccinia virus with an exceptional safety profile serves as an advanced recombinant poxvirus vector in preclinical and clinical development of vaccines against infectious disease and cancer.
In this project, MVA serves as a highly versatile expression system to allow for stable synthesis of ZIKV proteins.
Results: We generated recombinant MVA candidate vaccines expressing ZIKV antigens compatible with clinical evaluation.
The target genes were cloned into MVA vector plasmids and introduced by homologous recombination into the MVA genome.
MVA-ZIKV-prME and MVA-ZIKV-C were genetically stable and replicated efficiently in CEF but not in human HeLa cells. Infections of human cell cultures with recombinant MVA demonstrated efficient synthesis of ZIKV prME and C proteins in cells non-permissive for MVA replication.
Conclusion: These recombinant MVA-ZIKV candidate vaccines await further testing in animal models in order to evaluate their immunogenicity and efficacy.

Analysis of proteolytic activation of the spike protein of MERS coronavirus

H. Kleine-Weber[1], M. Tarek[1], M.Hoffmann[1], S. Pöhlmann[1]

[1]Infection Biology Unit, German Primate Center, Göttingen, Germany

Keywords: MERS-CoV, S-protein activation, proteases

Background and objectives: Middle East respiratory syndrome coronavirus (MERS-CoV) can cause fatal disease in humans and poses a pandemic threat. Activation of the viral spike protein (MERS-S) by host cell proteases, such as TMPRSS2, furin and cathepsin L (CatL), is essential for host cell entry. However, the sites required for activation are incompletely understood. Here, we studied the role of specific cleavage sites on S protein-driven entry.
Materials and methods: We used rhabdoviral vectors, cell lines with endogenous expression of different proteases and protease inhibitors to analyze proteolytic activation of MERS-S. Moreover, PCR-based mutagenesis was employed to alter cleavage sites in MERS-S.
Results: We found that an intact S1/S2 site is important for S protein activation by TMPRSS2 but not by CatL. We further show that the S2' site is universally required for efficient host cell entry. Activation at the S1/S2 site requires an RXXR motif, while a single R residue is sufficient for activation at the S2' site. Mutation of proposed CatL site had no effect on host cell entry. Finally, residual entry observed upon parallel mutation of the S1/S2 and S2' site remained CatL dependent, indicating that CatL can employ auxiliary sites for S protein activation.
Conclusion: Our results reveal cell type-specific differences in protease choice for MERS-S activation and indicate a rigid sequence requirement for S protein activation by TMPRSS2 and potentially furin but not CatL.

Session 2: Risk Assessment and Epidemiology

October 18, 2018
09:00 – 10:30

Room Ballsaal
Chairs: Ard Nijhof & Stephanie Thomas

Can we predict the spatial origin of hantavirus infections using sequences derived from a generic screening PCR?

S. Weiss[1], B. Auste[1], D. H Kruger[1], J. Hofmann[1]

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Keywords: hantavirus, Puumala virus, phylogenetic analysis

Human hantavirus infections acquired in Germany are mostly caused by Puumala virus (PUUV) and recent outbreaks have been restricted to certain areas. Individual diagnosis is commonly based on serology as immunoblot, but nucleotide sequencing is required for molecular typing. The screening PCR is targeting the polymerase gene encoded on the large (L) segment of the tripartite genome, which is highly conserved across the hantavirus family. For phylogenetic analysis, the diverse small (S) segment encoding the nucleocapsid protein has been used before. Based on this, we have previously shown that there is a clear spatial distinction of PUUV clusters even among neighboring regions in Germany. Due to the high diversity of the S segment it can, however, be challenging to amplify nucleotide sequences. We investigated the potential of an L segment region, resulting from the screening PCR, to allow for spatial distinction of the virus origin, with the aim of facilitating future molecular epidemiological analysis.
Of 93 samples that tested positive in the screening (L) PCR 60 (65%) were also tested positive in the PCR of the S segment. Both phylogenetic trees resulted in comparable clusters revealing information about the geographical origin of the virus. In summary, analysis of sequences obtained from a diagnostic screening PCR allow for PUUV typing and can be used to assess the molecular epidemiology of the virus.

Molecular characterization and drug susceptibility of *Mycobacterium tuberculosis* from Eastern Sudan

Y. A. Shuaib[1,2,3],*, M. Merker[1], E. A. G. Khalil[4], U. Schaible[1], L. Wieler[3,5], M. A. Bakheit[6], S. El-Tiab Mohamed-Noor[2], M. A. Abdalla[2], E. Richter[1,7], K. Kranzer[1,8], S. Niemann[1]

[1]Research Center Borstel, Borstel, Germany; [2]College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan; [3] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany; [4]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan; [5]Robert Koch Institute, Berlin, Germany; [6]Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan; [7]Labor Limbach, Heidelberg, Germany; [8]London School of Hygiene and Tropical Medicine

Keywords: DST, genotype, tuberculosis, human, Sudan

Background: Tuberculosis (TB) is endemic in Eastern Sudan with an incidence of 275 per 100000 population. Data on genetic diversity of *Mycobacterium tuberculosis* and Multi drug resistant (MDR) are missing in this area. Therefore, in this study we focused on investigating drug susceptibility and population structure of *M. tuberculosis* in Eastern Sudan

Methods: A total number of 383 sputum samples were collected from Kassala, Port Sudan, and El-Gadarif hospitals from June to October 2014 and from January to July 2016. The samples were decontaminated and cultured into MGIT and onto Löwenstein-Jensen and Stonebrink slants for mycobacterial growth. Real-time PCR was conducted to verify the sensitivity of TB diagnosis using light microscopy in the study area. In addition, line probe assay, ITS gene sequencing, spoligotyping, MIRU-VNTR typing and whole generation sequencing (WGS) were also conducted.

Results: Culture of the specimens revealed growth of mycobacteria from 51.2% (196/383) of all samples. The majority of the isolates (n=171) were *M. tuberculosis* while the rest were either *M. intracellulare* (n=14) or mixtures of *M. tuberculosis* and *M. intracellulare* (n=11). Any drug resistance was detected in 22.6% (39/177) of all of the M. tuberculosis isolates while MDR was detected in 10.2% (18/177). The use of molecular techniques showed that 73.4% of the isolates belong to lineage 3 (Delhi/CAS).

Conclusion: There was an evidence of MDR transmission and acquisition. Lineage 3 (Delhi/CAS) isolates are responsible for causing the majority of TB cases.

Chikungunya in Germany: Where and when could it happen?

C. Beierkuhnlein[1], N. Tjaden[1], F. Englmeier[2], K. Nagels[2], S. Thomas[1]

[1]Department of Biogeography, University of Bayreuth, Bayreuth, Germany; [2]Chair of health care management and health services research, University of Bayreuth, Germany;

Keywords: arborviruses

Background and objectives: Formerly considered a mostly tropical disease, chikungunya is already found in Southern Europe. With its vector *Ae. albopictus* starting to spread across the country and demonstrating the ability to survive German winters, the public health system needs to be prepared.
Materials and methods: We use a species distribution model to identify areas climatically suitable for the vector. We analyse global flight passenger patterns and national imported incidence to find areas where introduction of the virus is likely. There, we use an epidemiological model to calculate the basic reproductive ratio R_0, followed by an economic model to estimate the costs of a hypothetical chikungunya emergence.
Results: Baden-Württemberg, the Saarland, Hesse and North Rhine-Westphalia (NRW) show the highest numbers of climatically suitable counties for an establishment of the vector. Baden-Württemberg, Rhineland-Palatinate and NRW have the highest suitability values, with further suitable areas in Bavaria. Similarly, high numbers of travellers returning from endemic areas are primarily reported from the South and West of Germany (Frankfurt, Munich, Rhein-Ruhr Area). The assessed medical costs during the acute phase are relatively low compared to the indirect costs caused by absenteeism.
Conclusion: The preparedness of the public health system can be improved by projecting the likelihood of possible new vector-borne disease hazards arising in Germany.

Evaluation of the zoonotic potential of H18N11 virus variant tested in the ferret model

M. Gorka[1], D. Hoffmann[1], K.Ciminski[2], M. Schwemmle[2], M. Beer[1]

[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany; [2]Institute for Virology, University Medical Center Freiburg, Germany

Keywords: Influenza A virus, bat influenza, zoonotic potential

Influenza A viruses (IAV) are important zoonotic pathogens that cause epidemic outbreaks in birds, swine and other mammals. In 2012 and 2013 two influenza A-like virus genomes were found in little yellow-shouldered fruit bats (*Sturnira lilium*) in Guatemala and flat-faced fruit bats (*Artibeus planirostris*) in Peru, provisionally designated as H17N10 and H18N11.
Conventional IAV hemagglutinins (HAs) bind canonical sialic acid-containing receptors. In contrast, biochemical and structural studies indicated that influenza A-like H17 does not. In fact, H17 and H18 HAs are unable to bind and hemagglutinate red blood cells, and are therefore atypical HAs. Whether or not these viruses are able to infect further mammalian species including the model species for human influenza pathogenesis: the ferret is currently unknown.
By reverse genetic techniques a H18N11 virus was generated. Passaging *in vitro* (canine cell culture) selected an H18N11 variant virus (rP11) with two mutations within the HA (K170R and N250S) and a stop codon in NA (G107X) protein. Ferrets were experimentally inoculated to check for the zoonotic potential of the variant virus.
Viral genome was detected in the upper respiratory tract, lung and brain but transmission to ferrets in direct contact was excluded. Out of 8 inoculated ferrets euthanized 7 days post infection or later 5 animals seroconverted. Therefore, we assume, the variant virus is poorly adapted to ferrets and does have a low zoonotic potential.

Multidrug-resistant zoonotic bacteria in companion animals from North-West Germany

U. Kaspar[1], K. von Lützau[1], A. von Lützau[1], A. Schlattmann[1], G. Peters[1], U. Rösler[2], R. Köck[1,3], K. Becker[1]

[1]Institute of Medical Microbiology, University Hospital Münster, Münster, Germany; [2]Institute for Animal Hygiene and Environmental Health, FU Berlin, Department of Veterinary Medicine, Berlin, Germany; [3]Institute of Hygiene, DRK Kliniken Berlin, Berlin, Germany

Background and objectives: Companion animals may act as a reservoir for multidrug-resistant organisms (MDRO) and transmit them to their human owners. While there are many reports on MDRO prevalence in humans and livestock, data for companion animals are rare. Here, we assessed the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-E) among these animals.
Materials and methods: Nasal, buccal and perianal swabs were collected from non-hospitalized horses (n=222), and dogs (n=192), cats (n=74) and rabbits (n=17) at veterinary practices in the Münsterland area. Swabs were streaked onto selective agars and suspended in enrichment broths. Identification of isolates was carried out by MALDI-TOF mass spectrometry. Susceptibility testing was done by VITEK followed by molecular confirmation and genotyping.
Results: MRSA were detected in two horses (0.9%), five dogs (2.6%) and two cats (2.7%) and assigned to *spa* types t034, t011 and t108 (all CC398), t843 (ST9) and t091 (ST7). While the *mecC* gene was detected only in one feline MRSA (t843), the remaining isolates harboured *mecA*. Nine horses (4.1%), seven dogs (3.6%) and one cat (1.4%) carried ESBL-E.
Conclusion: MDRO were found across all animal species with carriage rates similar to those reported for humans. This confirms the importance of the "One Health" approach for prevention of pathogen dissemination.

Zoonoses in Exotic Pets - Epidemiologic Survey on the Variegated Squirrel Bornavirus 1 in Captive Squirrels (Family Sciuridae) in Germany

V. Allendorf[1], K. Schlottau[1], V. Schulze[1], F. J. Conraths[1], R. G. Ulrich[1], M. Beer[1], T. Homeier-Bachmann[1]

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Keywords: VSBV-1, Epidemiology, Squirrel

Background and objectives: *Coxiella burnetii* is the causative agent of Q fever, which might result in an acute or chronic disease. Chronic Q fever develops years after infection and mainly manifests as endocarditis. How *C. burnetii* persist in patients is unknown. As tissue oxygen levels are low at sites of infection we investigate the influence of hypoxia on *C. burnetii* replication in macrophages (MΦ), their primary target cells.
Materials and methods: Murine bone marrow-derived MΦ were infected with *C. burnetii* under normoxic or hypoxic conditions.
Results: Under normoxic conditions *C. burnetii* replicates in MΦ. Exposure to 0.5% oxygen stabilized hypoxia-inducible factor 1α (HIF1α) and abolished *C. burnetii* replication in MΦ. Hypoxic-induced inhibition of *C. burnetii* replication was neither due to enhanced bactericidal MΦ activity nor linked to an altered intracellular trafficking. Our results rather indicate that *C. burnetii* enters a dormancy period under hypoxic conditions, which was dependent on HIF1α stabilization. HIF1α leads to reduced and delayed activation of STAT3. While constitutive active STAT3 rescued hypoxia-mediated impairment of *C. burnetii* replication, ablation of STAT3-signalling results in impaired *C. burnetii* replication under normoxia.
Conclusion: Hypoxia-induced stabilization of HIF1α impedes activation of STAT3 in MΦ, which in turn results in dormancy in *C. burnetii*. Therefore, hypoxic areas could provide a niche for *C. burnetii* persistence.

Session 3: Innate and Adaptive Immune Response

October 18, 2018
09:00 – 10:30

Room Zehlendorf
Chairs: Asisa Volz & Veronika von Messling

Interferon response is essential in containing human pathogenic Bourbon virus, a tick-borne Orthomyxovirus

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Keywords: interferon system, tick-borne pathogen, antiviral treatment

Background and objectives: A hitherto unknown viral pathogen was isolated in 2014 from a patient in eastern Kansas who died with high viremia shortly after disease symptoms developed. Additional cases with mild but also fatal outcomes have since occurred in the US. The novel virus, designated Bourbon virus (BRBV), belongs to the genus of tick-borne Thogotoviruses, *Orthomyxviridae*. Here we studied its pathogenicity in mice.
Materials and methods: Mice with defects in the interferon pathway were infected with BRBV. Virulence, survival and pathology were assessed. Furthermore, the potential of antiviral agents against BRBV was evaluated in cell culture and mice.
Results: Infected standard laboratory mice did not show any disease symptoms or signs of viral replication. In contrast the virus grew to high titers and caused severe pathology in mice carrying genetic defects in the type I and type II interferon (IFN) system. Tissue culture experiments revealed a high sensitivity of BRBV to IFN as well as to the antiviral agents ribavirin and T705-favipiravir.
Conclusion: We show that type I and II IFNs play a critical synergistic role in inhibiting BRBV. It stands to reason that the innate immune defence of the US patients were compromised. The CDC recommends supportive treatment for BRBV patients. We found that IFNα, Ribavirin and favipiravir have an antiviral activity against BRBV, thus providing potential therapeutic options for future cases.

GlykoViroLectinTools: novel mosquito and sheep C-type lectin receptor (CLR)-Fc fusion protein libraries to screen for CLR/pathogen interactions

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Keywords: innate immunity, C-type lectin receptors, Rift Valley Fever Virus

Zoonotic infections endangering livestock and human health represent a high socioeconomic burden worldwide. Rift Valley Fever virus (RVFV) transmitted via mosquitoes between ruminants and men threatens to spill from Africa to Europe. Understanding of its immune recognition in insects and mammalians requires a systems biology approach. C-type lectin receptors (CLR) are known to recognize a number of viral and nonviral pathogens. One of them, DC-SIGN, is a RVFV entry receptor in humans; yet, only little is known for the interaction of CLRs derived from mosquitos and sheep with RVFV. To this end, libraries of sheep and *Aedes aegypti* mosquito CLR-Fc fusion proteins were established. Respective CLR domains were fused to a human IgG1-Fc tag and the resulting CLR-Fc fusion proteins were transiently expressed in Chinese Hamster Ovary (CHO) cells. The functionality of the library was proven by ELISA-based binding studies with ligands known for mouse or human CLR orthologues. A RVFV ELISA coating protocol was established to allow screenings for CLR/virus interactions. Si-RNA based knock-down of DC-SIGN and Dectin-1 in monocytes was performed, with comparative experiments in mosquito and sheep cell lines conceived. The presented toolbox may lead to a better understanding of how RVFV interacts with innate immunity in the insect and animal hosts.

Funding of this project by the "Nationale Forschungsplattform für Zoonosen" (DLR/BMBF, Fkz. 01KI1724) is gratefully acknowledged.

Functional characterization of the interferon-induced antiviral factor tetherin of fruit bats

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Keywords: Fruit bats, tetherin, innate immunity

Background and objectives: Fruit bats harbor viruses that cause severe and often fatal diseases in humans, as exemplified by Ebola virus (EBOV) and Nipah virus (NiV). However, infected fruit bats do not show clinical symptoms. It remains elusive how fruit bats cope with viral infection but it has been speculated that their innate immune system is particularly adept at controlling viral replication. Here, we analyzed the interferon (IFN)-induced factor tetherin (BST-2) of fruit bats regarding its antiviral activity.
Materials and methods: Tetherin open reading frames were cloned from fruit bat cell lines and their antiviral activity was assessed in virus-like particle (VLP) release assays with directed tetherin expression. Further, the contribution of endogenously expressed fruit bat tetherin to IFN-induced control of spread of authentic vesicular stomatitis virus (VSV), EBOV and NiV was investigated.
Results: Fruit bat tetherin efficiently restricted VLP release and was insensitive to counteraction by the prototype tetherin antagonist HIV-1 Vpu. Similarly, fruit bat tetherin was resistant to antagonism by EBOV-GP, at least when high levels of the protein were expressed. Further, siRNA-mediated knockdown of tetherin expression in IFN-stimulated fruit bat cells augmented release of VSV, EBOV and NiV.
Conclusion: We identified fruit bat tetherin as a potent, IFN-regulated antiviral factor that may critically contribute to control of zoonotic viruses in their natural reservoir.
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MERS-Coronavirus strain with a fusion of two accessory genes isolated from a human patient

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Keywords: MERS-Coronavirus, innate immunity, accessory protein 4

Background and objectives: The Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic pathogen that causes nosocomial outbreaks. From a patient in Saudi Arabia (2014), we isolated strain MO4, carrying an in-frame fusion of the accessory genes ORF4a and ORF4b. ORF4a and ORF4b gene products interact with innate immune pathways. Here we studied whether the fusion of these genes (termed fused p4) involves changes of virulence.
Materials and methods: To compare strains in an isogenetic context, fused p4 was engineered in an infectious cDNA clone. As p4a normally prevents the induction of interferons by binding dsRNA, we performed dsRNA-binding assays. As p4b normally competes with NFκB for nuclear translocation, we analyzed subcellular protein localization by immunofluorescence. As nuclear NFκB induces *TNFalpha* expression, we measured *TNFalpha* gene expression by quantitative real-time RT-PCR.
Results: Compared to p4b, which was mainly localized in the nucleus, fused p4 was significantly localized in the cytosol. Fused p4 showed normal dsRNA binding suggesting normal function of the p4a portion. However, *TNFalpha* expression was upregulated 4-fold as compared to p4b, suggesting lack of interference with NFκB translocation. Virus growth was reduced against wild type by ca. 20%.
Conclusion: Against the assumption that MERS-CoV is selected for increased virulence when infecting humans, we identified a strain that has acquired a slightly deleterious mutation *in-vivo*.

Regions-specific regulation of type I interferon in the central nervous system protects from tick-borne encephalitis virus infection

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Keywords: interferon, tick-borne encephalitis, neurvirulence

Tick-borne encephalitis virus (TBEV) is an emerging arthropod-borne viral disease in Europe and Asia. TBEV is a member of the flavivirus family that causes a variety of severe symptoms like hemorrhagic fevers, encephalitis and meningitis in humans. Recent evidence has shown that innate immune response efficiently suppresses TBEV replication, but little is known about the role of type I interferon (IFN) response in neuroinvasion and neurovirulence.
We infected knock-out mice with Langat virus (LGTV), a naturally attenuated virus of the TBEV group and analyzed viral burden in the periphery and the central nervous system, type I IFN induction, brain infiltrating cells and inflammatory response.
We show that type I IFN is essential to control virus replication and spread in the periphery but in addition has a local antiviral effect in the brain. Interferon-β response is differentially regulated in distinct regions of the central nervous system influencing viral spread. Furthermore, CNS-resident cells showed differences in the induction of type I IFNs.
In summary, our data showed that local type I IFN response in the brain is essential to protect against virus replication in the brain and the local microenvironment of distinct brain regions is critical to determine virus permissiveness.

Murine fecal microbiota transplantation lowers intestinal *Campylobacter jejuni* loads and pro-inflammatory immune responses in secondary abiotic mice

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Keywords: Campylobacteriosis, colonization resistance, fecal microbiota transplantation

Background and objectives: Conventional mice are protected from *Campylobacter jejuni* infection by their distinct host specific gut microbiota composition. We here addressed whether peroral application of murine gut microbiota might be a therapeutic measure for lowering intestinal *C. jejuni* loads in vertebrates.
Materials and methods: Secondary abiotic mice generated by broad-spectrum antibiotic treatment were perorally infected with viable *C. jejuni*. One week later mice were stably colonized with 10^9 *C. jejuni* per g feces and subjected to oral murine fecal mcirobiota transplantation (FMT) on three consecutive days by gavage.
Results: Until two weeks post FMT, mean intestinal *C. jejuni* loads declined by approximately 5 log orders of magnitude. Remarkably, following FMT mice displayed less distinct large intestinal apoptotic and T cell responses that were accompanied by lower pro-inflammatory mediator concentrations in colonic *ex vivo* biopsies as compared to *C. jejuni* colonized mice without FMT.
Conclusion: Murine fecal microbiota transplantation might be considered an effective measure to lower intestinal *C. jejuni* loads in colonized/infected vertebrates including farm animals.

Session 4: Antimicrobial Use and Resistance

October 18, 2018
09:00 – 10:30

Room Steglitz
Chairs: Denise Rabold & Birgit Walter

Wastewater from a pig slaughterhouse as a reservoir for clinically relevant antibiotic-resistant pathogens and their dissemination into surface water

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Keywords: pathogens, wastewater, slaughterhouse

Background and objectives: Slaughterhouse wastewater is considered a hotspot for antibiotic-resistant pathogens (ARPs). The aim was to investigate the occurrence of selected clinically relevant ARPs in wastewater from a pig slaughterhouse and its municipal wastewater treatment plant (mWWTP).
Materials and methods: Wastewater samples (n=38) were taken along the production chain in a pig slaughterhouse (SH) in Germany with a slaughtering capacity of 11,000 pigs/day and its mWWTP (n=16). Samples were screened for ARPs using CHROMagar selective media. The final identification was done by MALDI-TOF-MS and resistance was confirmed by determining of MICs. Results: All wastewater samples including the outflow and the preflooder of the mWWTP were positive for ARPs. The majority of all strains (n=255) from the SH (86%) were represented by MRSA, *E. coli* and *A. baumannii* complex, whereas *E. coli*, VRE and *Klebsiella* spp. were the most widespread in the mWWTP (n=111; 80%). 15.7% of the strains from the SH were MDR, 1.2% carbapenem- and 3.0% colistin-resistant. The percentage of MDR strains from the mWWTP was 46.4%; of these, 3.4% carbapenem- and 5.9% colistin-resistant (negative for *mcr*-1/2/3/4). The tested MRSA (n=57) were LA-MRSA.
Conclusions: Wastewater from the investigated pig slaughterhouse is a reservoir for clinically relevant ARPs. They could still be found in the outflow and preflooder of mWWTP, which could pose a threat to human health, and needs to be further investigated.

Development of antibiotic resistance in fattening poultry

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Keywords: E. coli, antibiotic resistance, food chain, zoonosis

Background and objectives: Antimicrobial resistance in bacteria from the food chain is a major public health concern in Germany and elsewhere. Since 2011, antimicrobial use in animal production has decreased substantially in Germany. Consequently, it is expected that resistance rates decrease as well.
Materials and methods: We analysed data on antimicrobial resistance of *Escherichia coli* from the National monitoring on zoonotic agents and commensal *E. coli* along the food chain (animal production, slaughter, retail) during 2011-2016. Using R statistical software and KNIME analytics platform we applied Fisher's exact test in order to detect trends of resistance patterns in time.
Results: We found slight temporal decreases in multidrug resistance. For instance, between 2011 and 2016 the share of overall sensitive isolates increased from 13 % to 20 % in chicken meat at retail. However this was not observed across all substances tested and matrices considered.
Conclusion: Efforts in reducing antimicrobial use in meat production need to be continued. The monitoring of zoonotic agents as well as commensal bacteria keeps being an important tool to detect new resistance mechanisms as well as to assess trends in antimicrobial resistance rates. Furthermore, it is essential to evaluate the impact of any reduction strategy.

Characterization of a genetically encoded Tetracycline resistance determinant in *Chlamydia suis*

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Keywords: Chlamydia, tetracycline, antimicrobial resistance

TetC is the sole example of a genetically stable resistance acquired by an obligate intracellular bacterium. It is located on a genomic island in *Chlamydia (C.) suis*, a pathogen causing inapparent infections but also respiratory, intestinal and genital disorders in pigs. TetC consists of a central control region, a repressor tetR(C) and the resistance gene tetC and was shown to be in vitro-transferable from porcine *C. suis* to the closely related human pathogen *C. trachomatis* which is of particular public health concern.
The aim of our study was to characterize and compare *C. suis* isolates regarding their resistance to different tetracyclines and the genetic structure, regulation and functionality of their TetC resistance determinant. 14 selected isolates tested PCR-positive for the tetC resistance gene, but showed a different degree of susceptibility to tetracycline with MIC values varying from 1 to 8 µg/ml. To elucidate these differences, we investigated the functionality of the control region and tet(C) in a heterologous setting. First, the control region activity of six isolates was analyzed in a plasmid-encoded reporter gene system in *Escherichia coli*. Despite containing several different point mutations, the control regions of all isolates were functional. In a second heterologous expression trial, tet(C) from the six *C. suis* strains were shown to refer resistance to the *E. coli* host, demonstrating functionality of the resistance gene in all cases. Interestingly, isolates showed high MIC values (4-32 µg/ml) with tetracycline and oxytetracycline applied in pig farming, but seemed susceptible to doxycycline and minozycline (MIC 0.125-1 µg/ml) used in human therapy.

Identification of new mcr-3 colistin resistance gene variants in *Aeromonas* spp. of animal origin from Germany

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Keywords: colistin resistance, Aeromonas spp., mcr-3

Background and objectives: Colistin is one of the last treatment options for infections caused by multidrug-resistant Gram-negative bacteria. In this study, we screened *Aeromonas* isolates of animal origin for the presence of *mcr* genes and analysed them further by whole genome sequencing (WGS).
Materials and methods: A total of 479 *Aeromonas* spp. isolates, collected between 2005 and 2012, were screened by PCR for the presence of *mcr-1*, *mcr-2* and *mcr-3*. WGS was performed to analyse the *mcr*-positive isolates and genetic location of the *mcr* gene. Plasmid profiles were prepared and transformation/conjugation assays conducted for isolates with plasmid-borne *mcr* genes. MICs were determined by broth microdilution.
Results: Four of the 479 *Aeromonas* isolates (0.84%) were positive for *mcr-3*. Sequence analyses revealed the presence of four novel *mcr-3* gene variants, designated as *mcr-3.6* to *mcr-3.9*. The colistin MICs of the four isolates ranged from 4 to ≥128 mg/L. In three isolates the *mcr-3* gene variants were chromosomally located, while an *A. media* isolate carried two plasmids, of approx. 8 kb and 180 kb; the later encoding the *mcr-3.7* variant. A 16-fold increased colistin MIC in the transconjugant (8 mg/L) revealed the functionality of the *mcr-3.7* variant.
Conclusion: In this study, four novel *mcr-3* gene variants were identified in *Aeromonas* spp. isolates of animal origin. The new variant *mcr-3.7* was located on a non-typable plasmid that harbored also other resistance genes.

Analysis of *bla*$_{CTX-M-1}$-carrying plasmids from *Escherichia coli* isolates of diseased food-producing animals collected in the GE*RM*-VET resistance monitoring program 2008-2015

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Keywords: bla$_{CTX-M-1}$*, Escherichia coli, food-producing animals*

Background and objectives: The aims of the study were (i) to characterize CTX-M-1-producing *Escherichia coli* isolates from diseased food-producing animals and (ii) to analyse their respective *bla*$_{CTX-M-1}$-carrying plasmids.
Materials and methods: Among the 7,810 *E. coli* isolates from diseased food-producing animals collected in GE*RM*-VET (2008-2015) the ESBL gene *bla*$_{CTX-M-1}$ was identified in 352 isolates. Forty-eight representative isolates were analysed by phylotyping, XbaI-PFGE and multilocus sequence typing. The *bla*$_{CTX-M-1}$-carrying plasmids were transformed and characterized by conjugation, replicon typing and S1-nuclease PFGE. The transformants were subjected to antimicrobial susceptibility testing and PCR assays for the detection of resistance genes.
Results: The 48 isolates belonged to 30 sequence types and displayed unrelated XbaI-patterns. The *bla*$_{CTX-M-1}$ genes were located on plasmids of varying sizes (35-330 kb) of which 45/48 were conjugative. The most common incompatibility groups were IncN (n=16), IncF (n=14) and IncI1 (n=11). Mainly detected co-located resistance genes conferred resistance to tetracycline, sulphonamides, trimethoprim and gentamicin.
Conclusion: This study showed that (i) CTX-M-1-producing *E. coli* isolates from diseased animals are heterogenous and (ii) IncN, IncF and IncI1 plasmids are of importance in the dissemination of *bla*$_{CTX-M-1}$ genes. Furthermore, co-located resistances may facilitate the spread and persistence of *bla*$_{CTX-M-1}$-carrying plasmids.

High zinc oxide diets: effects on porcine intestinal *Escherichia coli* populations

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Keywords: Antimicrobial resistance, Escherichia coli (E. coli), zinc

Background and objectives: At present, zinc oxide-rich diets used in the pig production seem to be associated with intestinal persistence of antimicrobial-resistance together with a decrease in the total *E. coli* population. The aim of this work is a more analyses of a representative selection of *E. coli* isolates obtained from a high-zinc fed piglet group and a control group to unravel the mechanisms underlying the above mentioned observations.
Materials and methods: In total, 179 *E. coli* ("high zinc group": n=99; "control group": n=80) were screened for antimicrobial resistance and zinc tolerance by determination of the minimum inhibitory concentration (MIC). In addition, *in silico* whole genome screening 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).
Results: 47% of the *E. coli* representing the zinc-fed group showed resistance towards at least one antimicrobial agent vs. 38% in the control group. In total 39/179 investigated *E. coli* demonstrated a MIC of 512 µg/mL for $ZnCl_2$, ("high zinc group": n=37). The latter isolates where associated with phenotypic resistance towards tetracycline and trimethoprim/sulfamethoxazole, with corresponding genes located on a plasmid of 100kb size.
Conclusion: Further analysis of factors affecting zinc tolerance in *E. coli* is warranted including Zinc-induced stress response by comparative transcriptomic analyses and the influence of certain resistance-carrying plasmids with respect to distinct genetic backgrounds.

Session 5: Selected Plenary Talks of the Research Network of Zoonotic Infectious Diseases

October 18, 2017
11:00 – 12:30

Room Ballsaal
Chairs: Christian Drosten & Martin Blume

Promising options to combat campylobacteriosis - lessons learned from peroral gut microbiota transplantation and application of defined molecules in murine infection models

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Keywords: gut microbiota transplantation, polyphenolic compounds, anti-inflammatory effects

Our previous studies revealed that i.) Conventionally colonized mice are protected from *Camplyobacter jejuni* infection and that ii.) Polyphenolic compounds such as resveratrol and curcumin exhibit potent anti-inflammatory effects in acute murine gut inflammation. Therefore, we first challenged secondary abiotic mice with *C. jejuni* by gavage and subjected stably infected mice to peroral fecal microbiota transplantation (FMT) derived from either human or murine donors. Interestingly, fecal pathogenic burdens could be lowered up to 5 orders of magnitude within 1 week post-FMT, whereas *C. jejuni* induced pro-inflammatory sequelae could be alleviated with more prominent effects upon murine as compared to human FMT. Furthermore, secondary abiotic IL-$10^{-/-}$ mice were perorally pre-treated with either resveratrol or curcumin and infected with *C. jejuni*. Whereas placebo control mice suffered from acute enterocolitis six days thereafter, treatment with either polyphenolic compound (with most distinct effects exerted by curcumin) resulted in ameliorated *C. jejuni* induced pro-inflammatory immune responses that were not restricted to the gut, but could also be observed in extra-intestinal including systemic compartments. We conclude that FMT and/or peroral treatment with defined polyphenolic compounds might be effective future options for treating and/or even preventing from *C. jejuni* induced inflammation.

An unexpected development – classical bornavirus BoDV-1 as lethal zoonotic pathogene

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Keywords: Bornaviruses, Zoonosis, Reservoir Hosts

Mammalian bornaviruses are now represented in two species of the novel genus Orthobornavirus: Mammalian 1 orthobornavirus (with the classical Borna disease virus 1; BoDV-1) and Mammalian 2 orthobornavirus (with the variegated squirrel bornavirus 1; VSBV-1). Bicoloured white-toothed shrews (Crocidura leucodon) were described as the persistently infected reservoir host for BoDV-1, and squirrels from South America or Asia are considered as potential reservoirs for VSBV-1. Both reservoir hosts stay clinically healthy with no or only very few mild alterations despite efficient virus replication and broad tissue distribution. First fatal human infections with VSBV-1 were reported in 2015, and VSBV-1 was classified as the first confirmed zoonotic bornavirus. Very recently, also the first confirmed human BoDV-1 cases could be identified and were further characterized. Two patients, who each received a kidney of the same donor, developed severe neurological symptoms and died after a few months. A third patient receiving the liver of this donor survived the infection with subsequent damages. Molecular detection methods and whole-genome sequencing, antigen staining and indirect antibody detection were performed and identified BoDV-1 as the responsible pathogen. Our study confirmed for the first time the zoonotic potential of BoDV-1, and also proved that it can be transmitted by solid organ transplantation leading to a potentially lethal disease in immunocompromised patients. The zoonotic potential was further supported by additional lethal BoDV-1-related encephalitis cases.

Antibiotic Stewardship in Veterinary and Human Medicine

R. Köck[1,2], A. Lübke-Becker[3], B. Walther[4], and the #1Health-PREVENT consortium

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Keywords: antibiotics, carbapenems, resistance

The emergence of multidrug-resistant microorganisms (MDRO) affects both human and veterinary medicine. Antibiotic Stewardship (ABS) is defined as „ a coherent set of actions, which promote using antimicrobials in ways that ensure sustainable access to effective therapy." The key components of interdisciplinary ABS concepts are: 1.) Developing, consenting and implementing local guidelines for diagnostics, infection prevention as well as calculated and targeted antimicrobial therapy, ii.) Active training of prescribers by audits and feedbacks, iii.) Single interventions such as de-escalation, dose optimization, switch to oral therapy or antibiotic restrictions, iv.) Surveillance of antibiotic use and MDRO, and v.) Assessment of quality indicators for the treatment of infections.
In human medicine, about 1,000 clinicians, microbiologists and pharmacists were trained as ABS experts until 2017. However, thorough establishment of ABS teams in all German hospitals is still hampered by a lack of political, financial and technical commitment.
In veterinary medicine, mandatory structures for implementing ABS are also pending. Within the #1Health-PREVENT consortium, ABS is evaluated in a project focusing on I.) The effect of peri-operative use of antibiotics on the equine microbiome, II.) Use of antibiotics among cats and dogs with urinary tract infection or pyoderma, and III.) Individual decolonization strategies for feline and canine carriers of MDRO.

Session 6: New and Re-Emerging Zoonotic Diseases

October 18, 2018
14:30 – 16:00

Room Ballsaal
Chairs: Andrea Rasche & Martin H.- Groschup

Experimental risk assessment for chikungunya virus transmission in Europe

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Keywords: Chikungunya virus, Aedes albopictus, Europe-wide risk assemement

Background and objectives: Chikungunya virus (CHIKV) is a mosquito-borne alphavirus, causing chronic arthralgia. The establishment of the Asian tiger mosquito *Aedes albopictus* in Southern Europe and the regular import of CHIKV by infected travelers has resulted in at least 5 local outbreaks of CHIKV in France and Italy. The ongoing spread of *Ae. albopictus* highlights the need for a comprehensive analysis of the Europe-wide spatial risk of CHIKV transmission.
Materials and methods: *Aedes albopictus* specimens from Germany and Italy were orally infected with CHIKV and kept for 2 weeks at 18°C, 21°C or 24°C.
Results: Analyses of mosquito saliva for infectious virus particles demonstrated transmission rates (TRs) of >35% independent of the *Ae. albopictus* population or temperature. European temperature data indicated a potential risk of CHIKV transmission for extended time periods, i.e. more than 40 days with preceding 14 days having a mean daily temperature >=18°C. This was shown for territories already infested by *Ae. albopictus* as well as for wide parts of so far not colonized areas in Central Europe.
Conclusion: Thus, the current risk of CHIKV transmission in Europe is not restricted by temperatures allowing extrinsic incubation of the virus, but rather by the vector distribution. Accordingly, all European countries with established populations of *Ae. albopictus* must implement respective entomological surveillance and monitoring systems, allowing suitable control measures.

Novel sandfly-associated phlebovirus with evidence of neutralizing antibodies in humans, Kenya

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Keywords: arbovirus, phlebovirus, sandfly

Background and objectives: Surveillance for arboviruses is poorly implemented in sub-Saharan Africa and programs often focus on mosquitoes and ticks as vectors of arboviral diseases only. Here we tested sandflies from a rural area in Kenya for infection with arboviruses and tested patients with fever-of-unknown-origin for virus-specific antibodies.
Materials and methods: Sandflies [n=6,434] were collected in Kenya. Virus isolation was performed on Vero cells. NPV was sequenced from cell culture supernatant using the Illumina MiSeq platform. Vertebrate pathogenicity was assessed in cell culture and newborn mice. Human sera were screened for antibodies against NPV by virus neutralization test.
Results: We detected a novel sandfly-borne virus designated Ntepes virus (NPV). NPV has a phlebovirus-typical tri-segmented genome [L (6436 nt), M (4454 nt) and S (1701 nt)] and is related to, but distinct from *Gabek Forest phlebovirus*. Cell cultures derived from humans, livestock, and wildlife were susceptible to NPV. Infections of newborn mice with NPV caused rapid and fatal illness. Specific neutralizing antibodies were found in 13.9% (26/187) of human sera taken at the site of isolation of NPV and a disparate site 600 km away.
Conclusion: We have identified a novel sandfly-borne human-pathogenic arbovirus which seems to be infect humans across Kenya. Further epidemiologic studies are needed to identify symptoms of disease in humans and to assess the distribution of NPV.
analysis will be discussed in context of outbreak investigations.

CRISPR-Forward screening approach to identify host cell factors required for bat influenza A-like virus (bat IAV) cell entry

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Keywords: Bat influenza A viruses, CRISPR Screening, receptor

Background and objectives: In 2012 and 2013, two novel influenza A-like viral genome sequences have been identified in Central and South America bat specimens and provisionally designated as HL17NL10 and HL18NL11. These bat IAVs harbor surface glycoproteins, hemagglutinin-like (HL) and neuraminidase-like (NL) that are highly divergent from conventional influenza A virus (IAV). Several lines of evidence suggest that HL plays the crucial role in cell entry. However, the putative receptor(s) of HL has not been identified yet.
Materials and methods: We performed a genome-wide CRISPR forward screening to identify the entry receptor of bat IAV. We further validated the identified receptor using reverse genetic approaches and gain of function experiments.
Results: We identified the MHC-II complex HLA-DR as proteinaceous entry receptor for bat IAV. We demonstrated that knockout of HLA-DR in bat-IAV susceptible cells blocked the entry of bat IAV, whereas the entry of other viruses was unaffected. The ectopic expression of HLA-DR or HLA-DR homologs from bat, pigs and chicken in non-susceptible cells conferred susceptibility to bat IAV infection. Remarkably, in mice, bat IAVs robustly replicated in the upper respiratory tract, whereas mice lacking MHC-II were resistant to the infection.
Conclusion: We identified HLA-DR homologs from multiple species as entry receptors for bat influenza virus, suggesting their zoonotic potential across species barrier.

Cowpox Virus - Host Interactions: Identification and Confirmation of Virulence Factors

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Keywords: Cowpox virus (CPXV), virulence, zoonotic infection

Cowpoxviruses (CPXV) are endemic in Western Eurasia. An extremely wide host range characterizes these viruses. Rodents, specifically voles, are the natural reservoir hosts. However, spill-over infections to accidental hosts, including rats, cats and humans, are reported frequently.

In order to evaluate the pathogenic potential of different CPXV strains, we sought to identify virulence factors for rodents. For this purpose, we used two different CPXV strains, one isolated from a pet rat (RatPox09), causing several human infections in Germany and France in 2009 and characterized as highly pathogenic in Wistar rats (mortality rate up to 100 %), and the laboratory reference strain Brighton Red (BR), with low pathogenic potential in the model species. Full genome-sequencing showed a sequence similarity of 92% between BR and RatPox09.

The genome of RatPox09 encoded six open reading frames (ATI, p4c, NMDAr, 7tGp, D7L and CrmE genes) additionally. To focus on the phenotypic correlates of these genotypic differences we used CPXV-BAC-generated knock-in mutants and chimeras of both viruses.

Since *in vitro* growth kinetics showed no marked differences between the generated CPXV mutants, we determined significant differences within the Wistar rat model and assessed both mortality and morbidity.

Taken together, the investigated genes most likely encode important rodent virulence factors of CPXV and genotypic differences are indeed directly related and additively correlated to pathogenicity.

Human pathogenic *Leptospira* in small mammals

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Keywords: Leptospira spp.*, small mammals, reservoir*

Numerous mammals worldwide, including domestic and companion animals, can be infected by leptospires, but rodents and other small mammals are considered as the main reservoir. Human leptospirosis outbreaks occur sporadically in temperate zones. For example, such outbreaks were reported in 2007 and 2014 in Germany. Our investigations focused on identification of the most commonly occurring *Leptospira* genomospecies, sequence types and their small mammal hosts in Europe and German military bases in Afghanistan.
Using conventional *lipl32*-PCR, 621 of 5,650 kidney samples were tested positive. *SecY*- and MLST-PCR-based analyses identified three genomospecies and eight sequence types (ST). Common voles in Germany and Spain were infected with *Leptospira kirschneri*. Furthermore, ST 110 was exclusively detected in common voles and field voles in Germany. Similarly, Norway rats in Europe were exclusively infected with *Leptospira interrogans* ST 17. In addition, leptospires were also identified in house mice at the military bases in Afghanistan. Therefore, results of our analyses confirm a broad geographical distribution of *Leptospira* in small mammals and suggest them to be of important public health relevance in human settlements.
Perspectively, we intend to design a diagnostic platform for rodent-associated pathogen chip-detection (RAPiD), which enables a comprehensive and timesaving molecular bed-side and pen-side diagnostics of *Leptospira* and other pathogens causing similar symptoms.

Neutralizing monoclonal antibodies and alpaca derived single domain antibody fragments against Rift Valley fever virus

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Keywords: antibodies, SNT, RVFV

Background and objectives: The mosquito borne Rift Valley fever virus (RVFV) causes large outbreaks affecting human and many vertebrate hosts throughout Africa and the Arabian Peninsula. To date, no drugs are approved for therapy of RVF in humans. One frequently discussed promising option is the application of neutralizing antibodies against RVFV infection.
Materials and methods: For generation of monoclonal antibodies (mab) BALB/c mice were immunized with E.coli expressed RVFV-Gn. Supernatants from generated hybridoma cells were initially screened by an inhouse ELISA for specific antibody secretion. Neutralizing activities of reactive mabs were revealed by using a serum neutralization test (SNT).
Furthermore a new protocol was established to generate single-domain antibody fragments (V_HH) against RVFV by immunization of alpacas with an inactivated RVFV MP-12 strain. Specific V_HHs were identified by phage display, followed by indirect immune fluorescence assays and finally checked for neutralizing activities.
The most promising V_HH was also expressed as a trivalent construct and tested as described above.
Results: One specific neutralizing mab was identified, which exhibited a synergistic neutralizing effect combined with a non-neutralizing mab. Likewise displayed the V_HHs as well as the V_HH construct neutralizing activity.
Conclusion: Antibodies with neutralizing activity against RVF were generated and will now be evaluated for in-vivo protection in RVFV challenge studies in animals.

Session 7: Public Health

October 18, 2018
14:30 – 16:00

Room Steglitz
Chairs: Sascha Al-Dahouk & Uwe Rösler

Activity and prevalence of pathogens in *Ixodes inopinatus* and *Ixodes ricinus* in Southeastern Germany

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Keywords: Tick-borne encephalitis, Ixodes inopinatus, transmission cycle

Background and objectives: *Ixodes (I.) inopinatus*, is a recently described tick species from the Mediterranean, which had been historically erroneously reported as *I. ricinus*. In 2017, populations of *I. inopinatus* had been detected for the first time outside of the Mediterranean. The medical importance of this new tick species is unknown so far.
Materials and methods: In 2017, ticks were collected monthly in a location sympatric for *I. ricinus* and *I. inopinatus*. Tick were identified morphologically to species level and individually tested by PCR for TBE virus and rickettsiae.
Results: A total of 415 *I. inopinatus* and 579 *I. ricinus* were collected from March to October 2017 and tested. *I. inopinatus* activity did not interfere with *I. ricinus* activity. All (994 specimens) *I. inopinatus* and *I. ricinus* were tested individually for *Rickettsia* spp. and tick-borne encephalitis (TBE) virus. 29/415 (6.9%) *I. inopinatus* and 48/579 (8.3%) *I. ricinus*, tested positive for rickettsiae. Two *I inopinatus* (2/415; 0.5%) and two *I. ricinus* (2/579; 0.3%) specimens tested positive for TBE virus.
Conclusion: This is the first report comparing activities of *I. inopinatus* and *I. ricinus*. Furthermore, it is showed that *I. inopinatus can* carry TBE virus and *Rickettsia* spp., as *R. helvetica* and *R. monacensis*. Further vector competence studies are needed to elucidate the medical importance of this new tick species in Central Europe.

Areas with high hazard potential for autochthonous transmission of *Aedes albopictus* associated arboviruses in Germany

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Keywords: Aedes albopictus, viraemic travelers, dengue/chikungunya

Background and objectives: Intensity and extent of transmission of arboviruses, has increased markedly over the last decades. Autochthonous transmission of dengue and chikungunya by *Aedes albopictus* was recorded in southern Europe where the invasive mosquito was already established and viraemic travelers had imported the virus. As *Ae. albopictus* populations now show a spreading tendency into Germany, we look at which counties are most likely to see negative effects.
Materials and methods: We use data from the RKI Survnet database to map where the chikungunya and dengue incidence rate of viraemic travelers has been highest in the past. We build an environmental niche model to show climatic suitable regions in Germany for an establishment of the vector now or in the near future. These two factors together are then used to identify counties that require raised attention in terms of mosquito control, surveillance and monitoring.
Results: Freiburg im Breisgau, Speyer and Karlsruhe are the German cities with the highest likelihood of autochthonous transmission of *Aedes albopictus*-borne arboviruses. In addition, 8.8 million people live in regions considered to show an elevated hazard potential assuming further spread of the mosquito.
Conclusion: Beside the Upper Rhine Valley, the Lower Rhine area in North Rhine-Westphalia requires special attention. Here, both incidence rates and climatic suitability for the vector are high, and infrastructure for mosquito surveillance, monitoring and control is widely missing.

Reduction of *Escherichia coli* cell numbers by colicins

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Keywords: Escherichia coli, colicins, bacteriocins

Background and objectives: Food can be contaminated with *Escherichia* (*E.*) *coli* along the food chain as a result of hygienic deficiencies. The use of colicins provides a possible way to reduce the number of *E.coli* in food. Colicins are ubiquitous in the environment, thus naturally present in food. Colicins are antimicrobial proteins produced by *E. coli* to lyse closely related strains. The aim of this study was to compare the cell number reductions of *E. coli* DH5α by different colicin concentrates at 4°C.
Materials and methods: The supernatants of the *E. coli* strains 2116 and 2147 were concentrated by tangential flow filtration with a molecular weight cut off of 10 kDa (first concentrate). The concentrates were further purified by ammonium sulphate precipitation and dialysis against PBS (second concentrate). The overnight culture of *E. coli* DH5α (10^7 CFU/ml) was incubated 1:1 with the bacteriocin concentrate at 4°C. At different time points CFU was determined by drop plating and activity units (AU) by spot assay of a 1:2 serial dilution series on the overlay agar of *E. coli* DH5α.
Results: The first concentrate of *E. coli* 2116 showed a reduction of *E. coli* DH5α cell number by up to 6 log units at 4°C immediately after the addition of the concentrate.
Conclusion: First results indicate that colicins could be an effective agent for reducing *E. coli* numbers in food under cooling temperatures. Further colicin concentrates will be tested.

One Health Interventions of Vétérinaires sans Frontières Germany in the current anthrax outbreak in South Omo Zone, Ethiopia

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Keywords: Anthrax, One Health, drought

Background and objectives: Vétérinaires sans Frontières Germany (VSFG) is operating under the One Health approach for eight years in several projects in South Omo, Ethiopia, where Anthrax is endemic. Regular outbreaks related to climate change, particularly to drought periods, affect this region. The main objectives of VSFG's project activities in anthrax management are (1) preventing new outbreaks, (2) protecting public health from livestock-sourced infections and soil contamination during an outbreak and (3) raising awareness for zoonotic diseases in pastoral communities.
Materials and methods: Among other activities, newly trained Community Animal Health Workers (CAHWs) and VSFG staff support the Government in the official livestock vaccination campaigns, herd isolation, innoxious removal of infected animals and, most importantly, they function as two-way information sources between affected communities and government during outbreaks. Moreover, members of the affected communities conduct rangeland rehabilitation and fodder production through cash for work activities.
Results: The activities have contributed to the protection of the soil from recontamination and pastoralists and livestock from new infections, hence strengthening livelihoods.
Conclusion: A holistic integrative approach, where all stakeholders, including the members of the communities, CAWHs, public sector institutions and civil society organizations work together is crucial to achieve health and livelihood.

Genotypic and phenotypic characterization of *Listeria monocytogenes* originating from food production environments

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Keywords: Listeria monocytogenes, antimicrobial resistance, genetic diversity

Background and objectives: *Listeria monocytogenes* (Lm) is a major food borne pathogen that is prevalent in the natural environment and colonizes various animals. Ready-to-eat products are frequently contaminated during food processing and present an important source of human infection. Thus, the aim of our study was to analyse the geno- and phenotypic diversity of Lm originating from German food production plants.
Material and methods: Whole genome sequencing was performed for 93 Lm isolates (Illumunia MiSeq) for genetic characterization. Minimum inhibitory concentrations (MIC) of relevant biocides and antibiotics were determined.
Results: The Lm isolates belonged to 23 MLST clonal complexes. Virulence factors like *Listeria* pathogenicity islands 1, 3 and the untruncated gene *inl*A were identified in 65, 10 and 75 isolates, respectively. The stress survival islet that contributes to enhanced survival in harsh environments was present in 38 isolates.
15 isolates were tolerant to benzalkonium chloride and 13 of these strains contained known genetic tolerance markers. Antibiotic resistance to daptomycin (100%), tetracycline (39%), meropenem (8%), ciprofloxacin (5%) and rifampicin (1%) was determined with variable genetic background.
Conclusion: The majority of isolates contained virulence factors necessary for human infection. In addition, several isolates possessed geno- and phenotypic traits that may contribute to enhanced survival in food production environment.

Health economic assessment of a possible chikungunya transmission in Germany

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Keywords: chikungunya, economic burden, climate change

Background and objectives: The threats of vector-borne virus infections like chikungunya are likely to emerge in Germany due to the progressing climate change. A possible transmission entails not only health risks for humans but also economic consequences for the health system. This work aims to estimate the related cost of illness and the development of assessments which can be applied to other clinically relevant vector-borne virus infections.
Materials and methods: We use a Markov model to analyse and project clinical and economic outcomes across German high risk areas. The acute and possible chronic course (chronic arthritis) of chikungunya as well as the corresponding transition probabilities are shown on the basis of a disease process model. To determine the newly infected cases the basic reproduction number is used based on a SIR model.
Results: During the acute phase the assessed medical costs are relatively low compared to the indirect costs caused by absenteeism. However the major cost drivers are both the indirect and direct costs of the chronic course. These account for about 80 % of total expenditure caused by an outbreak.
Conclusion: Modelling of specific health economic consequences are needed for informed and future orientated health policy decision making on both preventive strategies and outbreak management. With regard to chikungunya infections the modelling helps to design care processes that focus on the chronic sequela of the infection in a prioritized way.

Session 8: Pathogen-Cell Interaction

October 19, 2018
09:30 – 10:30

Room Zehlendorf
Chairs: Anja Lührmann & Imke Steffen

Bacterial nucleases as growth factor during bacterial co-infections

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Keywords: neutrophils, co-infections, host-pathogen interaction

Pasteurellaceae - like *Actinobacillus pleuropneumoniae* (*A.pp*) and *Haemophilus (H.) influenzae* - can cause severe pneumonia. Neutrophils transmigrate to the site of infection, release neutrophil extracellular traps (NETs) and entrap invading pathogens by DNA-fibers. Some bacteria are known to release nucleases to escape from NETs. Here, we investigated if NETs are part of the host-pathogen interaction in *A.pp* and *H. influenzae* infections. Furthermore, we analysed if nucleases derived from co-infections with *Streptococcus (S.) suis* and *Staphylococcus (S.) aureus* contribute to the disease.

We analysed bronchoalveolar lavage fluid (BALF) and lung tissue of *A.pp*-infected pigs for NET-markers and nuclease activity by microscopy, ELISA and biochemical assays. Growth experiments in presence of neutrophils and nucleases derived from bacterial co-infections were conducted.

We found numerous NET-markers *ex vivo*. Surprisingly *A.pp* grows in presence of NETs and even better in presence of degraded NETs. This phenotype was confirmed for *H. influenzae*. Nucleases of *S. suis* and *S. aureus* during co-infection significantly increased the growth of *A.pp* in presence of porcine NETs. *A.pp* itself does not produce extracellular nucleases.

We conclude that *A.pp* and *H. influenzae* benefit from free available DNA-fragments derived from NET-degradation during co-infections. This suggests a kind of co-evolution of porcine and human pathogens with zoonotic agents in the presence of host innate immune cells.

Hypoxia-induced citrate limitation results in upregulation of *C. burnetii* persistence genes

I. Hayek[1], F. Fischer[1], J. Schulze-Luehrmann[1], K. Dettmer-Wilde[2], R. Lang[1], P. Oefner[2], S. Wirtz[3], J. Jantsch[4], A. Lührmann[1]

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Keywords: C. burnetii, hypoxia, persistence

Background and objectives: *Coxiella burnetii* is the causative agent of the zoonotic disease Q fever. Apart from acute Q fever, around 2-5% of *C. burnetii* infected humans will develop chronic Q fever, which mainly manifests as endocarditis years after exposure to the pathogen. The clinical picture of chronic Q fever suggests that *C. burnetii* establishes a persistent state. Yet, information about the trigger(s) and site(s) of persistence is rare.
Materials and methods: Murine bone marrow-derived macrophages (MΦ) were infected with *C. burnetii* under normoxia (N) or hypoxia (H).
Results: Under N, *C. burnetii* replicates in MΦ and fails to induce robust accumulation of HIF1α. Exposure to H, however, stabilizes HIF1α which is essential for inhibiting *C. burnetii* replication. Mechanistically, HIF1α impairs the activity of STAT3, reduces the intracellular citrate level and thereby prevents *C. burnetii* replication. This suggests that H areas might provide a niche for *C. burnetii* persistence. To clarify this assumption, we analyzed the expression of persistence genes in H MΦ infected with *C. burnetii*. In fact, under H, genes encoding for proteins such as RNA polymerase sigma factor RpoS, (p)ppGpp synthetase RelA and RNA polymerase-binding protein DksA were upregulated as compared to infected N MΦ. This upregulation is lost upon replenishing the H MΦ with citrate.
Conclusion: Our data suggest that persistence of *C. burnetii* is triggered by hypoxia-induced citrate limitation.

Generation and characterization of synthetically-derived bat mumps virus

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Keywords: mumps virus, viral entry, replication

Background and objectives: Mumps is a highly contagious disease with usually mild symptoms caused by human mumps virus (hMuV). In 2012 viral RNA of a virus with phylogenetic relatedness to hMuV was detected in African bats (batMuV). In the absence of an infectious isolate, recent efforts to characterize batMuV were based on directed expression of viral glycoproteins, or chimeric MuVs harbouring batMuV F and HN. Although these studies provided initial insights in the functionality of batMuV F and HN, important aspects such as the host range, replication competence or virulence of remained elusive.
Materials and methods: We generated recombinant batMuV and investigated (i) its replication in cells of human, bat and non-human primate (NHP) origin, (ii) its neurovirulent potential and (iii) the interference with the IFN and TNF-α signalling pathway.
Results: BatMuV is able to replicate in cells of human, NHP and bat origin. For most of the cell lines analyzed - especially bat cells - the viral replication of batMuV was more efficient compared to a hMuV strain. *In-vivo* studies assumed that batMuV exhibits neurovirulent properties. Viral replication was mostly unimpaired by an ongoing IFN response suggesting that batMuV codes for proteins that have an antagonistic function against IFN-stimulated genes.
Conclusion: With the generation of recombinant batMuV we provide a suitable tool for further studies investigating the interplay with the host immune response or serological studies.

A cell model to investigate ENaC-dependent sodium absorption and tight junction alterations in campylobacteriosis

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Keywords: epithelial sodium channel, tight junction, epithelial barrier

Background and objectives: The zoonotic human pathogen *Campylobacter jejuni* (*C.j.*) is a leading cause of gastroenteritis worldwide. *Campylobacter concisus* (*C.c.*), a close relative pathobiont that colonizes the human oral cavity and the intestine, can also cause diarrhea and gastroenteritis. *In vitro* studies revealed that *C.c.* induces apoptotic leaks and tight junction (TJ) changes in intestinal epithelial cells. Our present study focusses on the influence of *C.j.* and different *C.c.* strains on Na^+ transport through epithelial sodium channel (ENaC) in the colon.

Materials and methods: We employed HT-29/B6-GR/MR cell monolayer (HT-29/B6 cells transfected with gluco- and mineralo-corticoid receptors) to study the uptake of Na^+ by ENaC. The ENaC-dependent sodium absorption was recorded as amiloride-sensitive short-circuit current (I_{SC}) in Ussing chambers.

Results: Measurements revealed that infection with *C.j.* and distinct *C.c.* strains blocked the Na^+ transport via the ENaC by about 50%. In parallel, we also observed an increase in epithelial permeability towards fluorescein after infection.

Conclusion: Our *in vitro* studies revealed that the *Campylobacter*-infection blocked the ENaC-dependent Na^+ transport (Na^+ malabsorption) and promoted an increase in epithelial barrier permeability in the cell model to fluorescein (leak flux). For future directions, we employ this infection model to investigate the host cell signaling pathways in ENaC and TJ regulation.

Session 9: Novel Methods, Diagnostics and NGS

October 19, 2018
09:30 – 10:45

Room Steglitz
Chairs: Caroline Herr & Martin H. Groschup

Monitoring wildlife and their pathogens using flies

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Keywords: fly, mammal, pathogen

Background and objectives: By definition, zoonoses occur at human/wildlife interfaces. In many cases, the wildlife component of these interfaces is poorly characterized. This prevents properly estimating zoonotic risk since the presence of potential reservoirs can pass unnoticed. Here, we describe the use of blowflies, which feed on vertebrate feces and carcasses, to characterize mammalian communities and the pathogens that affect them.
Materials and methods: We trapped flies at >10 locations in sub-Saharan Africa as well as in Berlin. We performed metabarcoding analyses of their diet to reveal the underlying local mammalian communities. We also screened fly-derived DNA for mammalian-infecting bacteria and viruses and attempted to grow some of the detected pathogens.
Results: We were able to characterize local mammalian communities in all sampled environments, including tropical forests (high and low altitude rainforests and dry deciduous forests), savannahs and urban areas. We detected bacterial and viral agents infecting mammals. We also grew rainforest anthrax from flies, which allowed us to generate >50 whole genomes of this potentially zoonotic pathogen.
Conclusion: We conclude that fly-based characterization of human/wildlife interfaces represents a very efficient and cost-effective strategy to simultaneously collect information on wildlife and their pathogens. This tool is a promising complement to more traditional methods employed for interface surveillance.

Successful oral vaccination against HPAIV H5N1 with novel bat influenza A virus chimeras

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Keywords: vaccine, bat influenza, H5N1

There are no life vaccines available that would efficiently protect chickens against highly pathogenic influenza A viruses (HPAIV). Here, we present a new type of vaccine backbone, based on internal segments originating from bat influenza virus H17N10 carrying the immunogenic HA and NA segments from a low pathogenic AIV (LPAIV) of subtype H5N1 designated H5N1-bat. These chimeric viruses do not reassert with conventional influenza viruses, including HPAIV, due to incompatibilities of packing sequences and viral proteins.

Groups (n=10) of adult and day-old SPF-chickens were prime-boost immunized by oral application of a chicken-adapted H5N1-bat and subsequently challenged with a lethal dose of HPAIV H5N1 strain R65. Clinical scoring, virus detection and serology were used to analyse the course of the trial.

While seroconversion could not be detected in sentinels and individual animals before challenge infection, none of the chickens showed any clinical signs after immunization or challenge. In oral swabs and organ samples of infected animals no viruses could be detected in the vaccinated animals.

In conclusion, the oral immunization with the newly developed live vaccine candidate induced 100% protection and sterile immunity against a lethal dose of a HPAIV H5 challenge virus. Future studies will e.g. define the minimum protective dose and genetic stability of the H5N1-bat chimeric vaccine strain and the feasibility to generate live vaccines encoding H7 and H9 of LPAIV.

Exploring "big sequence data": De-novo detection of viral pathogens by a dynamic database approach

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Keywords: Next-generation sequencing, pathogen detection, big data analysis

Background and objectives: Emerging and re-emerging viral infectious diseases cause frequent threats to both human and animal health. The lack of diagnostic methods for these novel pathogens can lead to severe delays in detection and subsequently counteracting the disease. Diagnostic sequencing by unbiased next-generation sequencing is a key method for the detection and identification of pathogens and also allow the characterization of mixed infections. The amount of data in such metagenomics-oriented sequencing approaches is continuously growing, thus impeding comprehensive exploitation of the entire data. The interdisciplinary project DetektiVir developed a new approach by combining molecular nucleic acid-based virus detection by sequencing and customized serological diagnostics in a dynamic database.
Results: Central part is a novel diagnostic data hub that combines raw sequence reads and metadata with the results from taxonomic classification software in a low-code platform. The core system offers flexible interfaces and algorithms in a user-friendly environment, enabling analyses and evaluation of big sequencing data across different samples. While implementing, we were already able to discover novel viruses like an ovine picornavirus and a new paramyxovirus with putative zoonotic potential.
Conclusion: Exploring metagenomic sequencing data is an ongoing challenge. Using a dynamic database approach we were able to pave the way to earlier identification of pathogens.

Persistent virus infection in mosquito derived cells

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Keywords: insect-specific virus, mosquito, persistent

Background and objectives: Many insect cell lines are persistently infected with insect-specific viruses (ISV) often unaware of the researchers. Most of the times, these infections result from persistent infections of the mosquito eggs/ embryos that had been used for the production of the corresponding cell lines; however cross-contaminations between cell lines are also possible. In light of recent findings showing the possibility of interference between arbovirus and ISV infections it is important to be aware of ISV infections in cell lines.
Materials and methods: A combination of small RNA sequencing, electron microscopy and PCR is used to investigate persistent virus infection in commonly used aedine cell lines.
Results: We describe the detection of Entomobirnavirus, Culex Y virus (CYV) in Aag2, U4.4 and C7-10 cells. In contrast to U4.4 and C7-10, CYV infection in Aag2 cells is hypothesized to be a cross-contamination as CYV-free Aag2 cells exist. Interestingly, the magnitude of PCR-positivity is variable among cell passages and leads to irregular detection via electron microscopy. Besides, other ISVs belonging to different virus families were detected as well using the small RNA sequencing and PCR approach in mosquito-derived cells.
Conclusion: Taken together, these results show that most of the common used mosquito cells harbor at least one persistent infection with an ISV and are often persistently infected with several different

Gene expression and signaling pathways in the human colon mucosa during *Campylobacter jejuni* enteritis

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Keywords: campylobacteriosis, epithelial barrier, bioinformatics

Background and objectives: We conducted RNA sequencing (RNAseq) in colon biopsies from *Campylobacter jejuni* (*C.j.*)-infected patients. Aim of this clinical observation study was to measure gene expression in patients' mucosae and identify host signaling pathways during infection.
Materials and methods: RNAseq from human colon biopsies was done by Illumina HiSeq2500. Demultiplexing and mapping was done by CASAVA and STAR. Statistical analyses were performed by R software. Upstream regulators and pathways were identified by Ingenuity Pathway Analysis (IPA).
Results: In *C.j.* infection, 5400 genes were down- or up-regulated. From this gene expression data we determined the signaling pathways by IPA. Upstream regulators are ranked upon their differentially expressed downstream targets. The most significant upstream regulator with *activating* pathways was LPS (P=3.2E^{-66}); the second most significant was IFN-γ signaling (P=3.95E^{-44}). Other top upstream regulators were all epithelial barrier-affecting TH1/TH2 cytokines like TNF-α or IL-13 (P=3.2E^{-41} or P=1.4E^{-30}). The top upstream regulator with *inhibiting* pathways was calcitriol (P=9.0E^{-25}).
Conclusion: RNAseq and upstream regulator data confirmed prior observations and provide a big amount of information along the *leaky gut* concept. The regulation pattern with inhibition of calcitriol-dependent pathways in the *C.j.*-infected mucosa led to the prediction that supplementation with vitamin-D may counter-regulate the inhibition of target genes.

Session 10: Epidemiology and Ecology of Zoonotic Infections

October 19, 2018
09:30 – 11:00

Room Ballsaal
Chairs: Sandra Junglen & Helge Kampen

Epidemiology of tick-borne encephalitis in Germany in 2017

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Keywords: Tick-borne encephalitis, epidemiology, Germany

Background and objectives: Tick-borne encephalitis (TBE) is the most important arboviral disease in Central Europe. The epidemiology of the tick-transmitted TBE virus is poorly understood and the numbers of human cases varies more than twice in different years. Detecting new trends in the epidemiology of TBE in Germany will help to focus surveillance and prevention methods in the mainly involved regions.
Materials and methods: Human cases of TBE were registered with the support of the Robert-Koch-Institute and the Bavarian Federal Office of Health and Food Safety. Human cases were contacted and followed to identify the location of tick infestation and ticks were collected to isolate and characterize the TBE virus strains.
Results: In 2017 a total of 485 human cases of TBE were registered, the second highest number reported since 2001. In Bavaria 234 human cases were reported the highest number since 2001. Bavaria and Baden-Württemberg were the federal states with the highest reported human case numbers. However there are clear spatio-temporal differences on a local level. In the northern parts human cases decreased while in the southern part along the Alpian mountains an increase was seen.
Conclusion: TBE is a disease with changing epidemiology in Germany. Following these changes will help to focus resources and prevention efforts to the main involved regions. In some districts TBE has to be named an emerging viral infection as there is a clear pattern of emergence.

Tick-borne encephalitis virus goes into the mountains

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Keywords: Tick-borne encephalitis, mountains, eco-epidemiology

Background and objectives: Tick-borne encephalitis (TBE) is the most important arboviral disease in Europe. In Germany the main endemic regions are located in the south. During the last years there is a clear change of TBE natural foci to higher altitudes. So far it is unclear from where these virus strains are invading and whether they exhibit phenotypic characters adapted to mountainous conditions.
Materials and methods: Human TBE cases were contacted and asked for the place of tick infestation. Ticks were collected from the respective locations in mountainous areas and tested for TBE virus. From positive ticks TBE virus strains were isolated and genetically and phenotypically characterized testing growth under different conditions.
Results: In 2017 there was clear increase of human TBE cases in mountainous areas of the Alpian mountains and of the German Central Mountains (States of Thuringia, Saxony). So far five newly established natural foci could be identified in the Alpian Mouintains. All foci are located in mountainous areas at altitudes between 650 and 700 m. The virus strains are genetically not closely related.
Conclusion: This study provide for the first time provides data on the phylogeny of TBE virus in newly established foci in mountainous areas. These phylogenetic data imply that TBE virus strains from different areas are introduced by different ways and find ecologically appropriate conditions to establish new natural transmission foci.

Field work of the AECO project - Vector biology of *Aedes albopictus* and eco-bio-social drivers for effective vector prevention & control in cooler ecoregions

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Keywords: One Health, dengue, Aedes albopictus

Background *Aedes albopictus* and *Ae. aegypti* mosquitoes they transmitting three of the most important arthropod-borne dengue, chikungunya and zika viruses belong to the world's most feared mosquito species with high social and medical importance.
Objectives The eco-bio-social research plan of the research group AECO focus on (i) the vector biology of the highly invasive mosquito *Aedes albopictus* and (ii) eco-bio-social aspects influencing vector prevention & control practices along a climatic gradient in a dengue and chikungunya epidemic country (Nepal). The overall objective of the AECO research project is to explore eco-bio-social determinants for development of integrative and community-based mosquito prevention and control measures in specific ecoregions of Nepal.
Results: After one year of AECO research and coming freshly back from the main field survey in Nepal, we will report the development of entomological and social tools in laboratory and field. Furthermore, we will present first results on vector biology (cold tolerance) and social research at different altitudes in Nepal. At the date of abstract submission, we perform a field test on optimizing egg collection and transport, a validation study on our social toolbox (validity of questionnaires, clustering of sample sites) and a training of entomological and social field teams for main entomological and social survey in September/October 2018.

Environmental occurrence of *Campylobacter* spp. in broiler farms and surroundings

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Keywords: campylobacter, tenacity, environment

Background and objectives: Emission of *Campylobacter* spp. into the environment has been known as major public health and economical concern. Nevertheless, the occurrence and tenacity of *Campylobacter* spp. in broiler farm surroundings is remained unclear. As part of the PAC-Campy consortium, we study the prevalence of *Campylobacter* spp. in broiler farms and their survival in surrounding areas.
Materials and methods: Campylobacter-positive broiler farms are selected by an initial screening (boot swabs and pooled faeces samples). These farms are sampled intensively by taking boot swabs, air samples and swab samples from various surfaces both inside the barn and in the environment. To measure the prevalence inside the barns pooled faeces are gathered. Sampling will take place during summer and winter time. At each farm two consecutive fattening periods and the efficacy of cleaning and disinfection are investigated. All Samples are processed based on DIN ISO 10272 for *Campylobacter* spp. and positive samples were further analysed with Mass spectrometry and multiplex PCR assay.
Results: The first screenings proved two investigated farms to be positive for *Campylobacter* spp. At farm A, all 4 barns and at farm B one out of four barns were tested positive. Mass spectroscopy and PCR identified *C. jejuni* in all positive samples.
Conclusion: The investigations will help to better understand the significance of *Campylobacter* spp. in the broiler farms surroundings for spread and re-entrance.

Clustering of hantavirus disease cases in a company in Lower Saxony, Germany, in December 2017, caused by intense bank vole infestation

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Keywords: Hantavirus, Outbreak, Zoonosis

Background and objectives: Since 2001, laboratory diagnosis of acute hantavirus-disease is notifiable in Germany, but until 2017 no institutional outbreaks have been documented in Lower Saxony (LS). In parts of LS Puumala orthohantavirus (PUUV) is endemic in bank voles and causes most of the human cases. In December 2017, a cluster of hantavirus-disease cases among staff members of a distribution company was notified from the administrative district Grafschaft Bentheim.
Materials and methods: We conducted an outbreak investigation including all employees of the company in a questionnaire survey asking for disease symptoms and potential exposures. Bank voles were trapped by use of snap traps early in 2018. Questionnaire data were analysed descriptively and rodents were tested for hantavirus RNA by S-segment-specific reverse transcription-polymerase chain reaction (RT-PCR).
Results: Seven employees participated in the survey. Three cases (1 f, 2 m, 30-48 years) fulfilled the case definition, one could be matched through the clinical-epidemiological link. The attack rate was 3/7. Five employees reported that they observed bank voles inside the company building. In total, 48 bank voles were trapped and 13 (27%) of them tested positive for PUUV-RNA.
Conclusion: The risk of hantavirus at the company site was confirmed through reservoir investigations. More hantavirus outbreaks are expected and could be confirmed through sensitising local physicians and enhanced molecular diagnostics.

Ticks and tick-borne pathogens in Sudan

Y.A. Shuaib[1], M.A. Abdalla[1], SE-T. Mohmed-Noor[2], A.M.A. Wd Elhag[3], Y.A.B. Ismael[3], G. Lemhöfer[4], S. Poppert[5], S. Schaper[4], G. Dobler[4,6], D.K. Bakkes[7], L.Chitimia-Dobler[4,6]

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Keywords: Sudan, Tick, Rickettsia

Background and objectives: From Sudan only very limited information about the occurrence of ticks and their carriage of pathogens is available.
Materials and methods: Ticks were collected from livestock (cattle, sheep, goats and dogs) in the Sudanese states of West Darfur, River Nile and Al-Jazeera in 2017. Ticks were morphologically identified and investigated for carriage of *Rickettsia* (R) by molecular methods
Results: A total of 1612 ticks were collected, belonging to the genera *Amblyomma* (A), *Hyalomma* (H) and *Rhipicephalus* (R), with a total of 16 species: *A lepidum* (57), *A variegatum* (4*), H anatolicum* (850), *H dromedarii* (30), *H impeltatum* (3) *H rufipes* (128), *H truncatum* (3), *R annulatus* (1), *R bequaerti* (7), *R bergeoni* (118), *R decoloratus* (30), *R evertsi* (328), *R guilhoni* (2), *R muhsamae* (1), *R praetextatus* (35), *R senegalensis* (13). The infestation per animal varied from 0 to more than 100.
Using a panRickettsial PCR 77/1612 (4.7%) ticks tested positive. This comprised 50/128 (39%) *H rufipes*, 8/30 (26.6%) *H dromedarii*, 12/57 (21%) *A lepidum*, 2/4 (50%) *A variegatum*, 2/30 (6.6%) *R decoloratus*, 2/328 (0.6%) *R evertsi*, and 1/13 (7.6%) *R senegalensis*. *H rufipes* and *H dromedarii* carried *R aeschlimannii*, while *A lepidum* carried *R africae*.
Conclusion: Ticks are of great relevance in Sudanese animal breeding and ticks may transmit *Rickettsia* to humans.

Poster Presentations

Poster Session Risk Assessment, Epidemiology and Modelling

R01

Phylogenetics of Tick-borne encephalitis virus in the upper Rhine region in France and Germany

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Keywords: Tick-borne encephalitis, phylogenetics, Rhine Valley

Background and objectives: Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Europe. It is mainly transmitted to humans by ticks. The upper Rhine valley and the Netherlands are thought to be the very western limits of TBEV distribution in Europe. The aim of our study is to study the phylogenetic relatedness of the circulating TBEV strains at the western borders of the TBEV endemic regions to better understand the mechanisms of distribution.
Materials and methods: Ticks were collected between 2016 and 2017 by flagging. TBEV was detected by RT-qPCR, isolated in cell culture from collected ticks and E genes were amplified and phylogenetic analyses were performed.
Results: At 12 sampling sites a total of 4,064 *Ixodes* ticks were collected in 2016 and 2017. The TBE foci were detected, two in Germany and one in France. The Minimal Infection Rates (MIR) ranged from 0,11 to 0,42%, respectively. Overall, the three newly described TBEV strains, isolated in the years 2016 and 2017 from the upper Rhine valley have no close phylogenetic relation and might rather form independent genetic clades.
Conclusion: In conclusion, we demonstrate, to our knowledge for the first time the phylogenetic relations of recent TBEV strains on both sides of the upper Rhine River. These genetic findings show that TBEV strains were introduced independently into the region, probably from Eastern Europe and that the Rhine River forms no geographic barrier for the spread of TBEV.

R02

Modular Process Risk Model of *Yersinia enterocolitica* in matured raw sausage products

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[1]Federal Institute for Risk Assessment, Berlin, Germany

Keywords: Yersinia enterocolitica, food processing, modelling

Background and objectives: In the European Union Yersiniosis ranks third in reported zoonosis with consumption of meat being the main cause. Our aim is to support assessing the risk emerging from the consumption of matured raw sausage products.
Materials and methods: We model the process chain of raw sausage products from slaughter over sausage production, maturation to storage. Hereby we derive quantitative estimates on *Yersinia* prevalence and load for supporting the risk assessment of these products. The model applies a modular approach on the process chain enabling the evaluation of interventions and hypothetical scenarios on the severity of *Yersinia* contamination. Required data are either gathered from scientific publications, zoonosis surveys and reports or created in own microbiological experiments
Results: We show the basic load of different sausage products with *Yersinia enterocolitica* and compare it to the effects due to differences in cooling, meat composition, interventions and consumption behaviour supporting risk assessment and providing starting points for the reduction of *Yersinia* contamination in raw sausage products.
Conclusion: Depending on the production process different sausage products show only minor decline of *Yersinia* contamination during maturation and are therefore capable of causing yersiniosis. Appropriate actions have to be taken to reduce this possibility.

R03

Increase of notified human brucellosis cases during the European refugee crisis, Germany, 2014-2016

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Keywords: brucellosis, Germany, refugee

Background and objectives: In 2000, Germany was officially declared free of bovine, ovine and caprine brucellosis, but human cases increased by 40% since 2014. We aimed to identify reasons for this increase.
Materials and methods: We analyzed symptomatic laboratory-confirmed cases notified 2007-16. Refugee status was captured since September 2015. Using official population data, we calculated risk ratios (RR) comparing incidences in refugees and non-refugees.
Results: Annual brucellosis notifications increased from a mean of 24 (2007-13, range: 18-28) to 42 (2014-16, range: 36-47, p=0.02). The proportion of males among cases increased (46% to 60%, p=0.017) and age decreased (median 49 to 41 years, p=0.009). Most frequently mentioned countries of infection shifted from Turkey (2007-13: 50%, 2014-16: 30%) to other Middle East countries (2007-13: 13%, 2014-16: 42%). Cases among refugees arriving in Germany 2015-2016 (80% male, median age 28) accounted for 9/44 (2015) and 15/36 (2016) of notified cases (RR refugees/non-refugees 2015-2016: 50, 95% CI: 31-80).

R04

Tenacity of *Leptospira* spp. on strawberries and in soil

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Keywords: Leptospira, survival time, strawberries, soil

Background and objectives: In 2007 and 2014 leptospirosis outbreaks were reported among strawberry harvesters in Germany. In two epidemiological studies the risk factors associated with leptospiral infection were, among others, contact with mud and eating strawberries. As little to no data are currently available, the tenacity of *Leptospira* spp. in these environmental matrices was investigated.
Materials and methods: A comprehensive literature search was conducted on the tenacity of *Leptospira* spp. in environmental matrices. Further, a study was designed to determine the survival time of *Leptospira* spp. on strawberries and in soil under different conditions including temperature, humidity and incubation time. Also, optimal conditions for washing and detecting of *Leptospira* on spiked strawberries were developed.
Results: The evaluation of the review data showed the ability of *Leptospira* spp. to survive in soil for several days depending of its moisture. Recovery of *Leptospira* spp. from spiked strawberries was most effective by shaking the whole strawberry in a flask with EMJH medium for 10 minutes. Preliminary data show a survival time of *Leptospira biflexa* serovar Sao Paulo up to 8 hours with the measured humidity up to 56%rh - 60%rh and a temperature of 25-27°C on strawberries.
Conclusion: *Leptospira* spp. are able to survive on strawberries and in soil depending on moisture as a major survival factor.
Conclusion: We confirm the importance of rodents as host of this zoonotic bacterium. In addition, we could also show that *Francisella tularensis* can be detected in certain close range hotspots.

R05

Prevalence and distribution patterns of potentially human pathogenic *Vibrio vulnificus*, *Vibrio cholerae* and *Vibrio parahaemolyticus* at coastal bathing areas of the German North and Baltic Sea from summer 2017 to summer 2018

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Keywords: Vibrio spp., pathogen, North and Baltic Sea

The occurrence of potentially pathogenic *Vibrio* (*V.*) species at coastal bathing areas of the German North and Baltic Sea steadily rising causing global warming. Due to those facts, the risk of *Vibrio* infections, especially wound infections, associated with recreational bathing in European coastal waters for visitors and residents increase.

To get an overview, this study monitors the seasonal and spatial distribution of *V. cholera*, *V. parahaemolyticus* and *V. vulnificus* at seven recreational bathing areas over one-year period starting in June 2017.

V. parahaemolyticus was found as dominant *Vibrio* species during all seasons round the study areas of the North Sea. In contrast, *V. vulnificus* was found as dominant *Vibrio* species during all seasons round the study areas of the Baltic Sea. Therefore, the salinity influence the *Vibrio* spp. composition, whereas the water temperature is the most important determinant to detect *Vibrio* spp. from the sampling areas. An explosive increase begins around about then degrease water temperature reaching the highest detection rates during the summer months until 10^4 KBE/100ml water and 10^6 KBE/100g sediment and decrease rapidly during the winter month. In sediments *Vibrio* spp. concentrations were up to three log higher than in water and were detected through the winter months indicating an important role for *Vibrio* ecology (VBNC).

The high detection rates of potentially pathogenic *Vibrio* spp. during the summer month should be regarded with care.

R06

Epidemiology of tick-borne encephalitis (TBE) in Germany – Update

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Keywords: tick-borne encephalitis, epidemiology, risk areas

Background and objectives: TBE risk areas in Germany are primarily located in southern states. Here, we provide an epidemiological update on this vaccine-preventable viral zoonosis.

Materials and methods: We analysed German TBE surveillance and vaccination coverage data, 2001–17. Risk areas are defined based on TBE incidence, using data of 4,569 cases with known place of infection.

Results: From 2001–17, the annual number of notified TBE cases fluctuated from 195 (2012) to 546 (2006). The proportion outside defined risk areas increased from 4% (2001–15) to 11% and 8% (2016 and 2017). In 2017, 156 districts in 7 states fulfilled criteria for TBE risk areas, 131 in Bavaria (BY), and Baden-Wurttemberg (BW). Ten first-time risk areas were located in BY, Saxony and Thuringia, all bordering known risk areas. From 2014–17, TBE incidence was highest among 50–59 year-olds and higher among males (2.7 cases/100,000 inhabitants in BY/BW) than females (2.2), but this difference was less pronounced than in the past. Hospitalization was reported for 87% of cases, increasing with age (p trend=0.003). Alimentary TBE transmission via infected goat milk occurred in one outbreak with ≥8 cases. Up-to-date vaccination was reported in 2.6% of cases.

Conclusion: TBE risk is highest southern states, with slow northward spread. The recent increase in TBE cases in long known risk areas underlines the need to improve stagnating vaccination coverage among the local tick-exposed population.

R07

Influence of mosquito microhabitat temperatures on the extrinsic incubation period of pathogens

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Keywords: mosquito, resting behaviour, microclimate

Background and objectives: The extrinsic incubation period (EIP) of vector-borne pathogens in cold-blooded insects directly depends on the environmental temperatures. Therefore, disease transmission models are generally based on data from weather stations. This data probably does not represent the actual temperatures present in the microhabitats of the vectors. Therefore, we studied the resting sites of mosquitoes and corresponding temperature conditions to identify the potential consequences for the EIP.
Materials and methods: In total, 288 artificial resting sites of different sizes (0.4 l, 76 l, 162 l) were attached to trees in different heights (0 m, 1.5 m, 4.5 m) at 16 anthropogenic study sites in Northern Germany. Each resting site was equipped with a data logger to record hourly temperature data. Mosquitoes were collected biweekly from April to October in 2016 and 2017.
Results: The numbers of collected mosquito specimens increased with increasing size of the resting site and decreased with the height of the placement. Resting sites can have a significantly higher temperature range compared to the nearest weather station. This results in shorting of the EIPs for pathogens like *Dirofilaria*, West-Nile virus or Usutu virus.
Conclusion: The study demonstrates that data from weather stations do not necessarily represent the temperatures in the microhabitats of vectors. Therefore, transmission models should include microclimate data to give a reliable estimation of the EIP.

R08

The strengthening of a biosafety system for the prevention of foot-and-mouth disease based on the recent situation in the Ukraine

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Keywords: foot-and-mouth disease, prevention

Background and objectives: Foot-and-mouth disease (FMD) is a viral zoonotic infectious disease. Animals that recover from FMD can get re-infected with a different type. For years, the Ukraine has had the status of a FMD-free country without vaccination. However, FMD cases have been reported in neighbouring countries. Our goal is to identify potential ways of FMD introduction endangering the FMD status in the Ukraine.
Materials and methods: We have employed analytic and statistical research (including descriptive statistics) to define the risk measures correlated to FMD (including cases reports).
Results: We've observed the epizootic disease situation over the time span of five years, and analysed viral serotypes prevalence as well as existing prevention measures. Based on this analysis, we've proposed improvements for said existing measures. The existing local technologies of vaccine production and a wide spectrum of vaccines have been included in the evaluation. We have evaluated the effectiveness of the available vaccines in regard to the presence and circulation of FMD serotypes as well as the conformity of the vaccine composition with the epizootic situation. Based on this, we've developed national recommendations for the prevention of FMD in the Ukraine.
Conclusions: The prediction of the potential spread of different FMD serotypes based on the evaluation of existing data is an integral part in establishing strategies to prevent the spread of FMD.

R09

Tick-borne encephalitis (TBE) in Southern Germany: Implementation of a case-control study to describe disease severity and identify risk factors

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Keywords: tick-borne encephalitis, disease severity, risk factors

Background and objectives: TBE is a viral disease transmitted by *Ixodes ricinus* ticks. Annually, ~320 (range 195–546) cases are reported in Germany. The high hospitalization rate (75%) among reportedly 'mild' cases suggests that statutory surveillance data incompletely capture true disease severity. We are launching a study to comprehensively assess symptoms and long-term sequelae, but also risk factors and TBE vaccine effectiveness. Our study is linked to partner projects in the TBENAGER research consortium, reflective of a One Health approach.

Materials and methods: In a case-control design, we will invite TBE cases reported in Bavaria and Baden-Württemberg from 2018 to 2020 and matched controls for telephone interviews. We will collect information on symptoms and health care utilization from cases and their physicians. Using a validated questionnaire (Neuro-QoL), we will estimate impact of TBE on quality of life acutely and after 1.5 years. We will also compare risk behaviours such as outdoor activities and use of tick protective strategies in cases and controls, including TBE vaccination to estimate vaccine effectiveness and identify vaccination barriers.

Results and Conclusion: We will report on the initial implementation of our study. Results will help to further characterize TBE disease burden regarding symptoms, quality of life and health service utilization. Identification of risk and protective factors and vaccination barriers will permit improved TBE prevention.

R10

Zoonotic diseases in the Republic of South Sudan: update, research needs and perspectives

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Keywords: vector-borne diseases, neglected zoonoses, haemorrhagic fevers

Background and objectives: South Sudan comprises a diversity of ecoregions from deserts to wetlands, and savannah to mountain rainforests. Scientifically most parts of this territory, which became an independent nation in 2011, have never been explored. Efforts to build a healthcare system have been hampered by armed conflicts since 2013 and an ensuing economic and financial crisis. Combined with droughts, this has led to humanitarian catastrophe marked by displacement, famine, and epidemics.
Materials and methods: We obtained data during 3 missions to South Sudan, from the Health Services Information System and NTD surveys of the Ministry of Health, publications and reports.
Results: While malaria causes the greatest burden of disease and most deaths in South Sudan, surveys revealed the presence of most WHO listed NTDs and zoonoses. Causes of past haemorrhagic fever outbreaks in South Sudan could not be identified. Improved laboratories, reporting and international cooperation were key in the response to a recent outbreak of Rift Valley fever.
Conclusion: The breakdown of essential services, rapid mass migration of people and livestock and difficult conditions in camps for displaced people have the potential to dramatically change the zoonotic landscape of South Sudan. Considering the economic and cultural importance of cattle, training young South Sudanese to conduct zoonosis research, prevention and control should go hand in hand with the humanitarian assistance.

R11

The tick *Dermacentor reticulatus* as driving vector for TBE virus transmission in a natural focus in Saxony, Germany

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Keywords: Tick-borne encephalitis, Dermacentor reticulatus, transmission cycle

Background and objectives: Tick-borne encephalitis (TBE) virus is circulating in so-called natural foci. In Central Europe the most common tick species serving as vector for TBE virus is *Ixodes ricinus*. Rarely, it is found in other tick species and, so far, only in *Dermacentor (D.) reticulatus* from Poland. Here we describe a new TBE focus in Saxony outside the defined risk areas where transmission seems to be driven by *D. reticulatus*.
Materials and methods: Ticks were sampled from autumn 2016 until April 2018 in a forest in northern Saxony. Ticks were pooled according to species, stage and sex. Testing for TBE viral RNA was done using the real-time RT-PCR and complete E gene sequences were generated after conventional RT-PCR. Results: TBE virus was detected in 12 flagged adult *D. reticulatus* pools and twice in *I. ricinus* nymph pools. Interestingly, TBE virus was not detected in *I. ricinus* during summer, when *D. reticulatus* was not active. Sequence comparison of the entire E genes of the isolated virus strains resembled each other with only single nucleotide differences. The most closely related viral sequences belonged to TBE virus strains from Poland and Neustadt/Waldnaab approximately 200 and 400 km east and south from the investigated focus.
Conclusion: This is the first report of TBE virus circulation in a natural focus where *D. reticulatus* and *I. ricinus* do occur sympatrically in nature but where *D. reticulatus* seems to be the main vector responsible for virus circulation.

R12

A documentation and assessment tool for the planning of intervention strategies against ESBL-producing *E. coli* in the broiler production in Germany

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Keywords: ESBL, Broiler, Modelling

Background and objectives: Extended-spectrum beta-lactamase (ESBL) -producing Enterobacteriaceae are an increasing problem in Public Health. Studies from all over the world showed high prevalence of these resistant bacteria in the broiler production as well as in chicken derived foods.
Materials and methods: We use a literature based approach to summarize possible intervention strategies and to populate a documentation and assessment tool for possible interventions against ESBL-*E. coli* in the broiler production. These interventions also target towards the reduction of antibiotic usage while ensuring animal health.
Results: Up to now 243 factors from eight different sources were identified as possible interventions in broiler fattening farms which were categorized into biosafety, internal and external biosecurity factors. Furthermore, 483 studies were investigated concerning the quantitative reduction of these resistant bacteria during processing of broiler chickens. However, these studies provide evidence for the effectiveness of the intervention measure only on the contamination level with total *E. coli* populations.
Conclusion: To prevent the occurrence and spread of ESBL-*E. coli* in the broiler production distinct intervention measures are needed. In the course of our joint research project we want to integrate the latest work of our collaborators using mathematical models. The developed tool should guide the farmer to take appropriate measures to reduce not only the presence of ESBL-*E. coli* but also the reduction of antibiotic usage.

R13

Who is at risk of occupational Q fever: new insights from a multiprofessional cross sectional study

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Keywords: Coxiella burnetii, occupational disease

Background and objectives: The zoonosis Q fever is caused by *Coxiella* (*C.*) *burnetii*. *C. burnetii* can be found in high numbers in the amniotic fluid, placenta and foetal membranes of infected animals. Human infection typically occurs by inhalation of aerosols. Q fever is recognized as an occupational hazard for individuals who are in regular close contact with animal birth products. Obstetricians are sometimes infected from human birth products. Despite this knowledge there are no systematic investigations of Q fever prevalence in occupational risk groups. The aim of study was to investigate the seroprevalence of *C. burnetii* in different occupational groups.
Materials and methods: 342 blood samples were screened for PhII *C. burnetii* antibodies by enzyme-linked immunosorbent assay. The study included samples of shepherds, cattle farmers, veterinaries, obstetricians, office employees and blood donors as control group.
Results: The highest seroprevalence was found in individuals with frequent animal contact (64-77%). There were no significant differences between cattle farmers, veterinarians and shepherds. The seroprevalence in people working in administration was lower but still significantly greater than the control. No obstetricians or midwifes tested positive.
Conclusion: Shepherds, veterinarians and cattle farmers have a high risk of *C. burnetii* infection. However, our study clearly proves that

there was no increased risk for people working in an obstetric department.

R14

Comparison resistant proportions in *Escherichia coli* for four common antibiotics in human and different animals: Data from national surveillance and monitoring for Antimicrobial Resistance (AMR) in Germany – German One Health Initiative (GOHI)

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Keywords: Escherichia coli, Antimicrobial resistance, One Health

Background and objectives: GOHI aims to strengthen cooperation and information exchange between human, veterinary und environmental medicines. Here, we analyze *Escherichia coli* (*E. coli*) data from three national surveillance and monitoring systems for AMR in humans and different animal population using harmonized threshold values.
Materials and methods: We used the reported *E. coli* data from 2014 to 2016 from *Antimicrobial Resistance Surveillance*–Network (ARS, Robert Koch Institute), *Zoonosis Monitoring* (German Federal Institute for Risk Assessment) and *GERM-Vet* (Federal Office of Consumer Protection and Food Safety) and compared the proportion of resistant isolates to common antimicrobial substances (ampicillin, ciprofloxacin, cefotaxime and gentamicin) in human clinical data (outpatient, normal station and ICU) with clinical and non-clinical data (slaughterhouse) from different animals.
Results: *E. coli* data showed highest resistance to ampicillin (40% - 55%) in humans and variability between animal populations with a range from 5% - 60%; to ciprofloxacin with 15%-20% in humans and with 2% - 35% range in different animals; to cefotaxime with range 5% -15% in humans and 1% - 30% different animals; and less than 10% resistant proportion to gentamicin for humans and different animals, except the diseased calves (ca. 30%).

Conclusion: The proportions of resistant *E. coli* isolates to commonly used antibiotics vary between humans and different animal populations.

R15

Modelling Mosquito-Borne Diseases in a Changing Climate: Current State-of-the-Art and Challenges in Cross-Disciplinary Research

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Keywords: Vector-borne diseases, modelling, climate

Background and objectives: Vector-borne diseases are on the rise globally. As consequences of climate change become evident, climate-based models of disease risk are of growing importance. For this, different concepts and approaches have emerged from different fields.
Materials and methods: Looking at the recent literature published in 2014–2017 from a European perspective, we review the current state-of-the-art in both mechanistic and correlative disease modelling, data driving them, vectors and diseases covered, and climate models applied to assess future risk.
Results: We find that modelling techniques have advanced considerably, especially in terms of using ensembles of climate models and scenarios. However, effects of extreme weather events, precipitation regimes and seasonality on vector-borne diseases are still poorly studied. Thorough validation of models is still a challenge and complicated by a lack of field- and laboratory data. On a larger scale, the main challenges today lie in cross-disciplinary and cross-sectoral transfer of data and methods.
Conclusion: Technical limitations aside, communication will be key for future improvements in modelling vector-borne diseases. Most models are primarily limited through (non-) availability of the necessary calibration data. Modellers need to make more clear what is needed to those who are in the position to produce the data in question.

Poster Session Pathogen-Cell interaction

P01

Ebola virus glycoprotein-driven entry requires cathepsin B/L activity irrespective of particle shape and target cell line

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Keywords: Ebola virus, glycoprotein, cathepsin

Background and objectives: Ebola virus (EBOV) poses a threat to public health. Priming of the viral glycoprotein (GP) is required for viral entry into target cells. Cathepsin (Cat) B/L prime GP *in vitro* but their expression is dispensable for EBOV spread in mice. The reasons for these discrepant observations are unclear. We seek to identify determinants of Cat B/L usage by EBOV-GP and to elucidate if Cat B/L activity is required for viral spread in primary target tissues.
Materials and Methods: Rhabdoviral vectors, EBOV-like particles and replication-competent VSV chimeras encoding EBOV-GP were used to evaluate Cat B/L dependence of viral entry. Cell lines and macaque lymph nodes ex vivo were used as targets for infection.
Results: We observed that entry of rhabdoviral vectors, EBOV-like particles and VSV-EBOV-GP into cell lines was invariably sensitive to Cat B/L inhibitors. Moreover, the requirement for Cat B/L activity for pseudotype entry was cell line independent. Calu3 cells express low amounts of Cat L and were not susceptible to EBOV-GP-driven entry. However, Cat L overexpression did not render these cells susceptible. Finally, VSV-EBOV-GP spread in nonhuman primate lymph nodes ex vivo was detected by RT-qPCR and it is currently under investigation if spread is Cat B/L dependent.
Conclusions: Our results indicate that Cat B/L-dependency of EBOV-GP-driven entry may not be determined by particle shape or target cell line and may not account for lack of infectious entry into Calu3 cells.

P02

Human Leaky Gut cell model for testing of potential therapeutics in experimental *Campylobacter jejuni* infection

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Keywords: campylobacteriosis, tight junction, electrophysiology

Background and objectives: The zoonotic pathogen *Campylobacter jejuni* (*C.j.*) is the most common foodborne cause for gastroenteritis worldwide. *C.j.* induces diarrhea and inflammation by invading the intestinal epithelial barrier (IEB). The *Leaky Gut (LG)* is characterized by an increase in permeability for macromolecules. After crossing the IEB or invading host cells, dysregulation of tight junctions (TJ), induction of epithelial lesions, and inflammation may occur.
Material and methods: We established an *in vitro LG model*, to develop a screening for protective or therapeutic substances. We perform molecule flux measurements, and analyze the inflammation signaling in co-cultures. The *LG model* serves also for confocal microscopy in *C.j.* infected cells, in order to analyze the initial phase of bacterial colonization (*live cell imaging*). For long-term infection, this model is also suitable to measure dynamic TJ changes, and visualization of bacterial translocation (via trans- or paracelluar pathway and/or *leaks*).
Results: Successful screened substances i.e. micronutrients (zinc), polyphenols (curcumin), and vitamins (calcitriol) show different protective and/or therapeutic effects against *C.j.* induced barrier defects.
Conclusion: This model is useful for characterization of pathogen-induced barrier dysregulations. By miniaturization, our model could also be suitable for high-throughput screenings and for other intestinal infections or food-associated diseases.

P03

SARS Unique Domain Regulates the Protein Level of Novel Antiviral Factor HDAC1 and Suppresses NF-KB Signaling mediated by MKRN2 and HDAC1

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Histone Deacetylase 1 (HDAC1) promotes degradation of antiviral factor p53 and negatively regulates nuclear factor p65-dependent NF-KB signaling. Both HDAC1 and p53 are targeted by E3 ubiquitin ligase Ring Finger and CHY Zinc Finger Domain Containing 1(RCHY1) which is a strong interacting partner of SARS Unique Domain (SUD). In this study, HDAC1 was for the first time characterized as an antiviral factor against coronaviruses. Overexpression of HDAC1 clearly reduces replication of pBAC-SARS-Luciferase replicon as well as viral replication of HCoV-229E and HCoV NL63. SUD augments the protein level of HDAC1 but it does not consequently enhance the antiviral effect of HDAC1. However, co-expression of SUD and HDAC1 leads to dramatic further suppression of p65-dependent NF-KB signaling which is negatively regulated by HDAC1. In addition, SUD interacts with E3 ubiquitin ligase Makorin Ring Finger Protein 2 (MKRN2). MKRN2 poly-ubiquitinates p65 and subsequently causes proteasomal degradation of p65. SUD causes dramatic reduction of p65-dependent NF-KB signaling via strongly stabilizing MKRN2. Thus, SUD suppresses NF-KB signaling through both HDAC1 and MKRN2 pathways. Moreover, SUD antagonizes antiviral factor p53 via increasing the protein levels of both RCHY1 and HDAC1 which lead to RCHY1- and MDM2-mediated p53 polyubiquitination and proteasomal degradation, respectively.

P04

Flow cytometry as a new tool to study *Coxiella burnetii* infection

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Keynotes: Coxiella, cell culture, flow cytometry, PCR

Coxiella (*C.*) *burnetii* is a Gram-negative, obligate intracellular pathogen that causes Q fever, a zoonotic disease with worldwide prevalence posing serious and often underestimated health problems in both humans and livestock. Q fever is self-limited flu-like illness, but can also cause interstitial pneumonia or hepatitis. Goats, cattle, and sheep are the primary reservoirs for human infection. Even though most transmissions to humans cause asymptomatic infections, they can also result in severe sequelae, such as endocarditis. Due to the obligate intracellular lifestyle of *C. burnetii*, the quantitation of infectious units (IFUs) or of genome equivalents (GEs) is routinely used for bacterial titer determination. A standard method for measuring GEs is quantitative real-time PCR (qPCR), while detection of IFUs can be performed by immunofluorescence microscopy using *C. burnetii* specific antibodies. Although both methods are widely used, they have critical limitations. Therefore, we established a new flow cytometric approach that permits fast, efficient and straightforward analysis of *C. burnetii* infections in cells. The method quantifies IFUs in a dose-dependent manner and allows for the specific detection of infection/replication-competent *Coxiella*. We show that flow cytometry of *C. burnetii*-infected cells ideally complement and extend advanced microscopic studies analyzing the size, morphology, virulence and intracellular localization of *C. burnetii* and their CCVs.

P05

Phosphorylation of serine 205 of influenza A virus NS1 protein as determinant of adaptation to a new host

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Keywords: influenza A virus, NS1, phosphorylation, adaptation.

Background and objectives: Influenza A virus (IAV) infection is a zoonotic disease that leads to epidemics and occasional pandemics. IAV NS1 is a multifunctional protein that was shown to be regulated by phosphorylation and linked to host adaptation. Circulating H1N1 viruses in humans show six amino acid changes compared to the swine origin 2009 pandemic (H1N1pdm09) virus, which eradicated previously circulating H1N1 (pre-pH1N1) viruses. Here, NS1 of pre-pH1N1 showed high prevalence of serine (S) at position 205 while pH1N1 exhibits an asparagine (N). Over the course of adaptation in humans, pH1N1 re-acquired S205 suggesting an important role in host adaptation. The major goal is to analyze the importance of NS1 S205 phosphorylation in viral replication and host adaptation.
Materials and methods: Reverse genetics were used to rescue pre-pH1N1 NS1 wild type and mutants with non-phosphorylatable glycine, aspartic acid to mimic constitutive phosphorylation as well as pH1N1 asparagine.
Results: Phospho-proteomic screening of NS1 identified S205 to be phosphorylated in pre-pH1N1 infection. Phosphorylation mutants replicated less efficiently compared to wild type. Furthermore, these mutants showed decreased amounts of viral proteins, which can be attributed to a diminished expression of viral mRNAs.
Conclusion: Tight temporal regulation of S205 phosphorylation is needed for efficient viral replication and might be re-gained as determinant of adaptation of pH1N1 to the human host.

P06

The host nonsense-mediated mRNA decay pathway seems not to restrict negative-strand RNA virus replication

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Keywords: Nonsense-mediated mRNA decay (NMD), Influenza A virus (IAV), virus-host coevolution

Background and objectives: Coevolution of viruses and their hosts resulted in multiple interaction networks between viral and host factors. Viruses evolved strategies to abuse host cells for their own multiplication and in turn, host cells gained strategies to restrict viral replication. Recently, a host RNA quality control mechanism called nonsense-mediated mRNA decay (NMD) was introduced as novel restriction factor against positive-strand (+) RNA viruses. However, so far there is no knowledge whether the NMD machinery carries host defence functions against negative-strand (-) RNA virus infections. Therefore, we investigated the effect of NMD on negative-strand RNA virus replication.
Materials and methods: Expression levels of NMD factors were manipulated or NMD was blocked by pharmacological intervention and the impact of these manipulations on growth of influenza viruses and respiratory syncytial virus (RSV) was analysed by multiple read outs.
Results: Manipulation of expression of the NMD factor UPF1 as well SMG5, SMG6 and SMG7 did not alter the replication of influenza viruses and RSV. Expression of viral mRNA and proteins as well as infectious particle production was not changed. In line with these findings, blocking NMD by a pharmacological approach did also not alter viral growth.
Conclusion: Our data emphasize that, in contrast to what was reported for (+) strand RNA viruses, the host NMD machinery seems not to affect replication of negative-strand (-) RNA viruses.

P07

Host response of *Campylobacter jejuni* mutants with altered invasion capability

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Keywords: Campylobacter*, pathogenicity, host response*

Background and objectives: *Campylobacter jejuni* belongs to the most important foodborne pathogens causing gastrointestinal infections in humans. However, the pathogenicity factors and human host cell responses related to the infection have not yet been adequately clarified. The aim of this study is to determine further *C. jejuni* invasion factors as well as the underlying regulation of host cell response to infection.
Materials and methods: The motility as well as the capacity of *C. jejuni* NCTC11168 mutants to invade human intestinal epithelial cells were investigated *in vitro*. Further, the expression of various mRNAs and lncRNAs, which might be related to cellular responses such as inflammation and apoptosis, were analyzed by means of RT-qPCR.
Results: One of the mutants had higher invasive and motility capacities while another mutant showed significantly decreased invasion and motility rates compared to the wild type. Expression analysis revealed several important coding and noncoding factors in host response to be dysregulated, e.g. reduced expression of a particular lncRNA that is involved in host cell apoptosis. Also, clear differences in the ability to induce IL6 were observed among the mutants.
Conclusion: The available mutations point out two so far uncharacterized genes of *C. jejuni* that not only play a role in invasiveness but can also influence the host response during infection.

P08

HtrA-dependent adherence and invasion of *Campylobacter jejuni* in human vs. chicken cells

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Keywords: Campylobacter jejuni, HtrA, intestinal chicken cells

Background and objectives: *Campylobacter jejuni* is one of the main causes for food-borne bacterial gastroenteritis. The intestine of poultry represents the major reservoir. Colonized poultry regularly does not show intestinal inflammation, whereas infected humans can suffer from severe gastroenteric symptoms. We have identified the secreted serine protease HtrA as an important virulence factor and chaperone in *C. jejuni*. We propose that the different capabilities of the bacteria to cause intestinal infection in human and poultry relies at least in part on HtrA function and its substrates.
Materials and methods: Various *C. jejuni* wild-type strain NCTC11168 and isogenic Δ*htrA* mutant were used for gentamicin protection assays to determine the adherence and invasion rates in human Caco-2 and chicken 8E11 cells.
Results: The wild-type *C. jejuni* was less adhesive and invasive in the 8E11 compared to the Caco-2 cells. A Δ*htrA* mutant showed decreased adherence and invasion in both cell lines. For adhesion, similar results were obtained using a proteolytic inactive $HtrA^{S197A}$ point mutant. Surprisingly, the same mutant showed reduced invasion in avian cells, but not in human cells. Instead, the invasion was comparable to the *C. jejuni* wild-type.
Conclusion: *C. jejuni* adheres and invades less efficient in chicken cells compared to human cells. HtrA is crucial for the adherence and invasion both in human and avian cells. Interestingly, the chaperone activity seems to be important for the internalisation in human cells and the protease activity for the invasion in chicken cells.

Poster Session Immune Response and Vaccines

I01

Identification of a distinct gut microbiota mediating colonization resistance against Campylobacteriosis in murine infection models

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Keywords: Campylobacteriosis, colonization resistance, gut microbiota

Background and objectives: Colonization resistance (CR) against *C. jejuni* (Cj) established by the gut microbiota of mice allows for developing novel therapeutic or preventive strategies to complement measures directed against Cj colonization and infection in farm animals and humans, respectively. We therefore surveyed the gut microbiota composition of mice with and without CR against Cj in order to identify distinct intestinal bacteria mediating colonization resistance against *Campylobacter* within the gut.
Materials and methods: The microbiota was analysed by deep sequencing (LGC Genomics) in mice with and without CR against C. jejuni. Briefly, CR against Cj is abrogated in mice treated with antibiotics such as ampicillin, ciprofloxacin, vancomycin, metronidazole or imipenem (single and in combination), in infant mice, and in mice harbouring a complex human microbiota. Conventional adult mice served as resistant controls.
Results: Analysis of the gut microbiota in mice with and without CR against Cj indicated that bacteria of the genera *Lactobacillus* and *Clostridium* might be involved in the establishment of CR against *Campylobacter*.
Conclusion: Further investigations within the PAC-Campylobacter consortium will reveal, if bacteria mediating CR against Cj in mice might be of use for prevention of *Campylobacter* colonization in poultry. Murine infection models will provide aid to validate possible therapeutic measures for the treatment of *Campylobacter* infection in humans.

I02

Polyphenolic compounds alleviate *Campylobacter jejuni* induced acute enterocolitis in secondary abiotic IL-10-/- mice

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

Background and objectives: Our recent intestinal metabolomic analyses revealed that phenolic compounds might be involved in mediating colonization resistance against *Campylobacter*. We here addressed whether peroral application of synthetic resveratrol or curcumin might be therapeutic measures for combating *C. jejuni* induced immunopathology.
Materials and methods: Secondary abiotic IL-$10^{-/-}$ mice were subjected to resveratrol or curcumin treatment via the drinking water starting four days prior peroral challenge with viable *C. jejuni* 81-176 strain (day 0).
Results: Six days post *C. jejuni* infection (p.i.), polyphenol-treated mice developed significantly less severe symptoms as compared to placebo controls - with most beneficial effects in the curcumin cohort. Particularly curcumin-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 curcumin-treated mice were approximately 7 log orders of magnitude lower at day 6 p.i. as compared to untreated controls with median fecal burdens of 10^9 CFU per g.
Conclusion: Due to its potent anti-*Campylobacter* and anti-inflammatory effects in murine infection models, curcumin represents a promising option for treatment and prophylaxis of *Campylobacter* infection and colonization in humans and farm animals, respectively.

I03

Immunomodulatory properties of the V and SH proteins of a bat-derived mumps virus

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Keywords: bat-derived mumps virus, host immune response

Background and objectives: Mumps virus (MuV), a member of the family *Paramyxoviridae*, causes the highly infectious disease mumps mostly resulting in mild symptoms but also affecting the CNS in rare events.
MuVs express one structural (small hydrophobic protein, SH) and one nonstructural protein (V protein) that are involved in modulating the host immune response by interfering with the TNF-α or IFN-α induction and signaling, respectively. In this study, we investigated the immunomodulatory effect of the SH and V protein of a bat-derived MuV (batMuV) that has been detected in an African flying fox. The amino acid sequence between the human and bat-derived MuV proteins is highly conserved with the exception of the SH gene that is known to be hypervariable among different human MuV strains.
Material and methods: The interference of batMuV V and SH with the TNF-α and IFN signaling pathways was investigated by (i) the infection of IFN-stimulated cells, (ii) the generation of recombinant MuVs lacking the expression of V or SH, (iii) the expression of MuV V and SH proteins and NF-κB or IFN reporter plasmids.
Results: BatMuV was able to replicate in IFN-stimulated cells of human and bat origin despite an ongoing immune response. The expression of batMuV V and SH led to a reduced activity of the NF-κB or IFN reporter plasmids.
Conclusion: Our data suggest that the batMuV V and SH proteins have a similar function compared to their human counterparts.

I04

The olfactory epithelium as a portal of entry in neonatal CNS infection

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Keywords: Listeria monocytogenes, neonatal meningoencephalitis, neonatal murine infection model

Background and objectives: Bacterial infections of the central nervous system (CNS) represent an important cause of morbidity in neonates. However, mechanisms of host susceptibility, route of infection and underlying mechanisms of neuronal inflammation remain ill-defined.
Materials and methods: Neonatal C57BL/6 mice were infected intranasally with *L. monocytogenes*. To determine bacterial dissemination, pups were sacrificed at various time points and organs were obtained for replica plating. Tissue tropism and immune responses were analyzed by immunohistochemistry, electron microscopy, flow cytometry and qRT-PCR
Results: We found that nasal, but not intragastric administration, led to early CNS infection in the absence of significant bacteremia. Immunofluorescence and electron microscopy demonstrated bacterial invasion of sensory neurons and supporting cells of the olfactory epithelium, followed by local growth within the *lamina propria*. *L. monocytogenes* subsequently spread along the sensory neurons of the olfactory nerve entering the brain tissue at the cribriform plate.

Subsequently, CNS invasion caused a significant induction of cytokines mediating intracranial inflammation as well as an influx of monocytes, monocyte-derived macrophages and neutrophils.
Conclusion: We propose an alternative portal of entry and route of infection for neonatal cerebral listeriosis and present a novel *in vivo* infection model to mimic the clinical features of late onset disease in human neonates.

Poster Session Antimicrobial Use and Resistance

A01

Characterization of Extended-spectrum beta-lactamase (ESBL)-harbouring *Escherichia coli* from diseased animals collected within the German GERM-Vet Monitoring

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Keywords: E. coli, ESBL, diseased animals

The occurrence of antibiotic resistances among bacteria causing infections in animals is problematic. In this study, *E. coli* isolates with extended-spectrum beta-lactamase (ESBL) resistance recovered from diseased livestock and companion animals were investigated regarding the distribution of beta-lactamase (*bla*) genes, their phylotypes and resistance patterns.
Antimicrobial susceptibility testing of 1158 *E. coli* from cattle, poultry, pig and companion animals obtained in 2016 within the German resistance monitoring program GE*RM*-Vet was performed. 102 isolates exhibiting an ESBL-phenotype were analyzed by phylotyping; *bla* genes were detected by PCR.
The ESBL phenotype was detected among isolates from pigs (5,7%), cattle (14,3%), poultry (2,6%) and companion animals (10%). Isolates were most frequently assigned to phylogroup A (28%), followed by B1 (23%), C (13%) and F (13%). First results revealed that $bla_{ctx\text{-}m}$ occurred within the majority of strains. Certain isolates harboured both, $bla_{ctx\text{-}m}$ and bla_{tem}. bla_{shv} variants were rarely found. Next to their ESBL resistance phenotype, 48 isolates exhibited a ciprofloxacin minimal inhibitory concentration (MIC) of >0,5µg/ml, 55 isolates had a MIC of >32µg/ml against Trimethoprim/Sulfamethoxazol and 7 isolates showed a MIC of ≥4µg/ml towards colistin.
It remains important to consider antibiotic resistances, especially those encoded on transferable genetic elements, which might lead to a co-selection of beta-lactam and other resistances.

A02

Antibacterial and immunomodulatory effects of *Balanites aegyptiaca* extracts: Novel therapeutic strategy against zoonotic bacterial infections?

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Balanites (B.) aegyptiaca (L.) Del. is a tree distributed throughout the Sudano-Sahielian region of Africa and the Middle East and South Asia. Distinct parts of *B. aegyptiaca* are used in traditional medicine e.g. the oil is consumed for headache and to improve lactation.
We investigated the antibacterial effect of extracts from mesocarp and bark against bacterial pathogens e.g. *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Secondly the immunomodulatory effect of the extracts on human granulocytes was analyzed.
Various bacterial clinical isolates were grown in presence of different concentrations of *B. aegyptiaca* extracts. The growth was monitored by measuring the optical density (OD) for 18 hrs in 30min intervals following quantification of colony forming units (CFU). Oxidative burst was measured by flow cytometry using dichlorofluorescein as fluorescence probe.
In contrast to the bark, the mesocarp extract significantly reduced the growth and surviving CFU of *S. aureus* strains as well as *E. coli* K1 (serotype O18:K1:H7) in a concentration dependent manner. Furthermore, the mesocarp and bark extracts significantly reduced intracellular ROS production of human granulocytes after 30 min of treatment in dose dependent manner.
In conclusion *B. aegyptiaca* extracts have antibacterial and anti-inflammatory effects. Further studies are in process to characterize the synergistic effects with antibiotics and to identify the active compound in the extracts.

A03

Characterisation of CTX-M-14 producing *E. coli* from German food samples

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Keywords: antimicrobial resistance, CTX-M-14, food

Background and objectives: The CTX-M-14 beta-lactamase is the second most detected ESBL determinant in *E. coli* of human infections. In samples of animal origin the enzyme is found less frequently. Here we present data regarding the occurrence and characterization of $bla_{CTX\text{-}M\text{-}14}$ harbouring *E. coli* from food samples.
Materials and methods: ESBL/pAmpC producing *E. coli* were isolated with selective media. The beta-lactamase was determined and $bla_{CTX\text{-}M\text{-}14}$ positive isolates were sequenced. Plasmids were further characterized through transformation, S1-nuclease PFGE and Inc group determination.
Results: A total of 13 CTX-M-14 producing *E. coli* were detected out of 404 ESBL/pAmpC producing isolates. The isolates were obtained from poultry products (8/13) or from cattle (beef/raw milk), whereas no CTX-M-14 producers were detected from pig products or vegetables. Five of the isolates belonged to the phylogenetic group B2 or D, which are associated with higher pathogenicity. All but one harboured the gene on plasmids, which often belonged to IncF. Inc group was not related to the animal origin of the food samples. Most (4/5) phylogenetic group A/C isolates belonged to ST744 harbouring the gene on IncF plasmids. SNP analysis revealed huge differences between isolates of different ST`s whereas isolates of similar ST`s (ST744; ST1266) form clusters.
Conclusion: CTX-M-14 beta-lactamases can be found in food with low incidence. The spread seems to be related to horizontal gene transfer as well as to two clonal lineages.

A04

Monitoring of Antibiotic Use in Cattle – Cross-Sectional and Longitudinal Data 2011 - 2015 in a German Livestock Sentinel

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Keywords: antimicrobial use, treatment frequency, regression modelling

Within the project VetCAb (Veterinary Consumption of Antibiotics), antibiotic usage data in German livestock are collected and evaluated. Based on a cross-sectional study in 2011, the project is continued as a longitudinal study "VetCAb-Sentinel" with ongoing participant recruitment and data collection since 2013. Data collection is based on official application and delivery forms, voluntarily provided by veterinarians and farmers. The database stores information about the number of animals treated, treatment date and duration, name and amount of the medicinal product used, indication and application route. In addition to evaluations of the treatment frequency (TF), temporal trends and the effect of the factors "farm size" and "region" on the TF were investigated, using multiple linear mixed and logistic regression models.

Results of the VetCAb study will be presented, focusing on trends in antibiotic consumption in dairy cows, calves and beef cattle during 2011-2015. It is shown that the median of the TF in calf (0.4→0.3) and beef cattle (0.2→0) holdings decreased between 2011 and 2015, whereas the median of the TF in dairy cow holdings remained almost constant. In the second half year of 2015, 25.1% of the calf, 11.2% of the dairy cow and 54.5% of the beef cattle holdings did not use any antibiotics at all. In calves the factors "time" and "farm size" and in dairy cows the factor "time" has a statistically significant impact on the antibiotic usage.

A05

Loss of Staphylococcal Cassette Chromosome mec V in Methicillin-resistant *Staphylococcus aureus* progeny

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Keywords: MRSA, secondary MSSA, large serine recombinases (ccr)

Background and objectives: *Staphylococcus aureus*, including Methicillin-resistant variants (MRSA) can be exchanged between companion or livestock animals and humans in both directions. As a result, humans and animals can suffer from a broad range of infectious diseases whereby increasing resistance against antibiotics results in severe therapeutic problems. In MRSA, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) harbour the *mec*A gene and cassette chromosome recombinases (*ccr*AB or *ccr*C), the latter catalysing the site specific genomic excision/integration of the elements. Loss of SCC*mec* elements occurs occasionally in MRSA, but the regulation of this process remains unknown.
Materials and methods: A high-throughput screening procedure was used to detect Methicillin-susceptible offspring from epidemic MRSA cultivated at different growth conditions. Progeny showing no growth on MRSA-chromogenic media were submitted to PacBio sequencing and analysed further.
Results: Comparative genomics revealed the loss of a complete SCC*mec* V element (16 kb) from the chromosome mediated by *ccr*. Of note, an additional complete SCC element harbouring resistance genes including *czr* and *tetK* together with a second variant of *ccr*C is still present in both, progenitor and methicillin-susceptible offspring.
Conclusion: To track the regulation and mechanisms involved in the integration and loss of SCC*mec* elements mediating methicillin-resistance, isogenic pairs of MRSA and secondary MSSA are needed.

A06

Prediction of phenotypical resistance of antimicrobial resistance from whole genome sequences

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Keywords: antimicrobial resistance, next generation sequencing (NGS),

Background and objectives:
In this study, we compared the antimicrobial resistance patterns of *E. coli* isolates from whole genome sequencing data with those obtained by phenotypic resistance testing.
Materials and methods: Genomes of 61 $bla_{\text{CTX-M-1, -14 or -15}}$ encoding *E. coli* obtained from food samples were sequenced on an Illumina MiSeq plattform. Assembled sequences were analysed by ResFinder3.0. The resistance phenotype of the strains was assessed by microbroth dilution according to CLSI guidelines and results were interpreted using the epidemiological cut-off values defined by EUCAST.
Results: The prediction of phenotypical resistance based on genetic data needs some experience. Especially for the prediction of aminoglycoside resistance, marker genes for the different antimicrobials streptomycin, kanamycin and gentamycin could be identified. It was possible to predict phenotypical resistance to aminopenicillin, trimethoprim, sulphonamides and tetracycline from genome data with a high correctness (>90%). Resistance to cefotaxime and fluoroquinolones was predicted consistently. *floR* indicates a resistance to florfenicol and chloramphenicol, whereas *cmlA1* and *catA1* resulted only in chloramphenicol resistance.
Conclusion: The prediction of phenotypical resistance based on WGS data is complex and needs further evaluation. In general, sequence data allow a more differentiated assessment of resistance as only some antimicrobials are tested phenotypically.

A07

A predominant ColE-plasmid prototype is associated with dissemination of the mcr-4 resistance gene in German *E. coli* isolates from food and livestock

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Keywords: Colistin, mobile, transmission

Background and objectives: Colistin is considered as highest priority critically important antibiotic, only used to treat human infections caused by multidrug-resistant Gram-negative bacteria. So far, seven different mobile colistin resistance-genes are known. Here, characteristics of *mcr-4*-positive colistin-resistant *E. coli* isolates from the national monitoring for antimicrobial resistance in zoonotic agents from the food chain were summarized.
Materials and methods: Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines and EUCAST epidemiological cut-off values. Resistant *E. coli* were subjected to *mcr*-PCR, S1-PFGE, MiSeq-sequencing (WGS) and bioinformatics. Transferability of *mcr-4* carrying plasmids was investigated by filter mating experiments.
Results: Up to now, 13 *mcr-4*-positive out of 756 colistin-resistant *E. coli* isolates, recovered between 2010 and 2017, were identified. Sanger sequencing revealed that two variants, *mcr-4.2* and *mcr-4.3*, are prevalent among these isolates. WGS showed that the isolates differ in their MLST-, sero- and fim-type. However, all of them carry a highly conserved ColE-plasmid prototype that partially differs in size and genetic composition.
Conclusion: Our findings indicate that *mcr-4* is sporadically distributed among colistin-resistant *E. coli* from food and livestock. However, further information on the biology and genetics are needed to assess the impact of this resistance determinant on public health

A08

Evaluation of different peri-operative antibiotic regimes in colic surgery on equine microbiome composition

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Keywords: equine microbiome, MRSA, ESBL-producing E. coli

Background and objectives: Veterinary clinics were identified as hot-spots for the distribution of multidrug-resistant bacteria (MDRB), such as methicillin-resistant *Staphylococcus aureus* (MRSA), ESBL-producing *E. coli* (ESBL-E) and *Acinetobacter baumannii*. The aim of this project is to comparatively investigate changes within the composition of the equine microbiome including occurrence of defined MDRB in horses receiving different peri-operative antibiotic regimes.
Materials and methods: Horses subjected to colic surgery were assigned to two groups, receiving either a combination of gentamicin/penicillin for five days or a single-shot prior to surgery. Faecal samples were taken directly at the time of admission to hospital (t0) as well as on days three (t1) and 10 (t3) after surgery. All samples were screened for MDRB using conventional microbiological diagnostics, while a second sample was stored at -80°C for profiling the 16S ribosomal RNA composition of the microbiome.
Preliminary results: So far, 15 of 54 horses admitted with signs of colic underwent surgical interventions. Considering all faecal samples obtained, 7% were ESBL-E-positive at t0, 28% at t03 and 50% at t10, confirming the urgent need for in-depth analysis of the equine microbiome after colic surgery. Conclusion: Results of this study are needed to provide a data-driven Antibiotic Stewardship program for horse clinics in order to significantly reduce the local burden of MDRB.

A09

Decontamination of a MRSA positive pig compartment under standard conditions

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Keywords: MRSA, decontamination, pig farming

Background and objectives: Complete and long-term decontamination of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig populations requires intensive cleaning and disinfection (C&D). Subject of this investigation was the analysis of decontamination of MRSA under standard C&D conditions.
Materials and methods: This research was performed on a conventional swine farm with flatted floors. Pig compartments were cleaned and disinfected by farm employees. Environmental contamination with MRSA was determined before and after C&D at 124 positions, localized in and outside the animal range. Nasal LA-MRSA carriage of the pigs was analyzed directly after housing and repeatedly during the weaning period.
Results: In total, 750 samples from surfaces, air and pigs were investigated. Of the environmental samples, 84% were MRSA positive before C&D. After C&D, on average 2-3% of these samples were MRSA positive; in- and outside the animals' range. During housing, 72% of the piglets were MRSA positive; at week six, all carried MRSA. Air sampling detected MRSA already within 30 minutes after housing of the previously MRSA negative stables.
Conclusion: The process of C&D under standard conditions considerably reduced environmental MRSA. The recontamination is due to MRSA positive animals. Hence, further studies should assess the preventive effects of measures (e. g. the use of competitive bacteria) that combine environmental decontamination and decolonization of animals.

A10

Effects of continuous fumigation and feed additive of *Lactobacillus* spp. on environmental bacteria in pig compartments

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Keywords: fumigation, lactobacillus, pig farming

Background and objectives: Prebiotics offers a new solution to reduce antibiotic use and to enhance animal health in pig farms.
Materials and methods: Lactobacillus (50,000 cfu/ml; 50 million cfu per day and compartment) was applied as an aerosol continuously in the stable air (compartment 1 and 3) and as a feed additive (2 and 3). Germ load on different surfaces were continuously determined in control (4) and treated compartments (1-3). Nasal LA-MRSA colonization of the pigs was analyzed directly after arrival in the stables and subsequently during the weaning period; environmental contamination of LA-MRSA was also determined.
Results: In total, 360 samples from surfaces and pigs were investigated. The total numbers of bacteria (total germs, staphylococcus and E.coli) in the treated compartments were significantly reduced. Lactobacilli were detected in all samples from surfaces in all compartments. These investigations had no influence on the animal health, daily weight gain and mortality in all groups. At arrival, all pigs carried LA-MRSA. This status was not changed; all pigs were still positive.
Conclusion: The use of lactobacillus in a continuously fumigation and/or as a feed additive generates an alternative microbiome in pig compartments with a reduction of pathogenic germs in the environment. These initial findings will be determined in further experiments with increased lactobacillus concentration and changes in the application method.

A11

Strategies to reduce antibiotic consumption in livestock: a survey involving German veterinarians and farmers

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Keywords: antibiotic resistance, antimicrobial stewardship, livestock

Background & Objectives: Legal federal provisions demand a major involvement of farmers to reduce the usage of antibiotics by means of the 16th amendment of the Medicines Act. As a result, farmers with the highest antibiotic usage in 6 months are obliged to submit a concept of strategies to reduce the antibiotic consumption (CSRAC). Veterinarians employed in regulatory authorities are responsible to evaluate those. A survey was conducted within the framework of the funded project "VetMAB II" to assess probable obstacles related to CSRAC and determine the demand for advanced training.
Materials & Methods: A Germany-wide online survey was conducted between the 3rd January and the 28th February 2018. The questionnaire was individually designed for the three target groups; (1) farmers, (2) veterinarians (livestock clinician), and (3) veterinarians in administration offices. Statistical analysis of anonymized data was performed using SPSS.
Results & Conclusion: In total, 508 participants filled in the questionnaire. Of those, 144 were farmers, 153 worked as livestock clinicians, and 211 were employed as veterinarians in administration offices. Vaccination and improved hygiene measures were estimated as the most effective strategies applied to reduce the extent of antibiotic consumption in livestock. However, farmers and veterinarians are uncertain about required content in CSRAC.

A12

Adaptation of *Staphylococcus aureus* to cationic surfactant antimicrobials from different classes

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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.

A13

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

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Keywords: Toxoplasma gondii, metabolomics, 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 fail to target these chronic stages of *T. gondii*.

To address this shortcoming, we screened a collection of antimicrobial compounds (MMV Pathogen Box) against tachyzoites and identified a number of growth inhibitory compounds. We now established another assay that allows us to test these compounds against otherwise drug-resistant, dormant and encysted bradyzoites in a plate-based assay. To identify the modes of actions of these novel antimicrobials we established a state-of-the-art LC- and GC-coupled mass spectrometry platform that detects hundreds of metabolites in an untargeted way.

In prospective experiments we will use this approach to compare global metabolic responses of the parasite to established inhibitors to those with unknown modes of action. Overall this project aims to leverage available compound libraries from various phenotypic screens to characterize mechanisms of the robustness of *T. gondii* tissue cyst metabolism in an untargeted way and facilitate development of new therapeutic approaches.

A14

Detection and Investigation of Extended-Spectrum Beta-Lactamase-producing Enterobacteriaceae in Wild Boar near Greifswald

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Keywords: ESBL, antimicrobial resistance, wild boar

Background and objectives: Antibiotic resistance poses a global risk for public health. Extended-spectrum beta-lactamase (ESBL)/AmpC-producing *E. coli* strains are widely distributed among pigs and can also be found in the surroundings of pig farms. However, it was unknown if ESBL/AmpC-producing Enterobacteriaceae are present in wild boar in Germany.
Materials and methods: The antimicrobial resistance of ESBL/AmpC-producing Enterobacteriaceae was investigated by testing faecal samples of wild boar shot close to Greifswald. A real-time PCR was performed for the detection of the beta-lactamase genes bla_{CTX-M}, bla_{SHV}, bla_{TEM} and CIT-type AmpCs in Enterobacteriaceae. Swab samples were taken, enriched and cultured on selective agar plates. Microbial identification and antibiotic susceptibility testing was conducted using the VITEK 2 system.
Results: Real-time PCR showed that 1 of 44 faecal samples was positive for ESBL resistance genes, while two faecal samples tested phenotypically cefotaxime-resistant in bacterial culture. These two samples were further analysed using the VITEK 2 system. One sample turned out to be *Hafnia alvei* while the other one was identified as *E. coli*. Interestingly, this *E. coli* strain tested ESBL-positive in bacterial culture and with the VITEK 2 test, tested negative by real-time PCR.
Conclusion: Therefore, in a next step, whole genome sequencing of the DNA of the *E. coli* strain will be conducted in order to optimize the qPCR and to characterize this *E. coli* strain.

A15

Decolonization of LA-MRSA positive fattening pigs in an alternative bedding environment

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Keywords: MRSA, decolonization

Background and objectives: The increasing occurrence of livestock-associated (LA) LA-MRSA requires a preferentially active counterstrategy.
Materials and methods: This research was performed on a conventional swine farm with open airing and straw bedding. Two different types of hygienic concepts were compared. Type 1: optimized cleaning and disinfection. Type 2: simple cleaning without disinfection. Nasal LA-MRSA colonization of the pigs was analyzed directly after arrival on the farm and subsequently during the fattening period; environmental contamination of LA-MRSA was also determined.
Results: At arrival, all pigs carried LA-MRSA. In follow-up screenings, pigs and environment in Type 1 stables showed several negative LA-MRSA reports after a few weeks, those animals farmed in Type 2 stables lost MRSA earlier. Whereas 72% of the pigs in Type 1 were MRSA negative, all pigs in Type 2 were MRSA negative at the end of the fattening phase after 16 weeks.
Conclusion: We hypothesize that these differences result from differences in the microbiome of the environment in Type 1 and Type 2 stables and could be supported by the straw bedding that facilitates bacterial competition. The eradication of LA-MRSA in swine farms via competitive bacteria will be investigated in detail in the #1Health-PREVENT project for a better understanding of this unknown bacterial influence and the development of an effective strategy against the spread of LA-MRSA.

A16

Quantification of Methicillin-resistant *Staphylococcus aureus* in broiler meat at retail in Germany.

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Keywords: MRSA, MPN, quantification

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) are widespread in animal populations and meat from livestock in Germany. The role of MRSA in food for the spread of LA-MRSA to humans is still considered negligible based on the close association of the occurrence of LA-MRSA in humans to contact with livestock. The causes for the discrepancy are not fully understood. One hypothesis is that the numbers of bacteria in meat are very low. The aim of this study was to detect MRSA in 215 samples of fresh broiler meat with skin taken at retail in Berlin and Brandenburg and to quantify MRSA in MRSA positive samples. We used a spatula method on selective agar plates for quantification and a "Most probable number" procedure for estimation of low numbers of MRSA. All isolated MRSA were confirmed by using MALDI-TOF and by detection of the *S. aureus* specific nuclease gene *nuc* and the resistance gene *mec*A. The highest estimated number of MRSA was 1100 MPN/g found in a wing sample. In 80.6 % of the 36 MRSA-positive samples MPN-estimates were lower than 10 MPN/g. Therefore, the numbers of MRSA in the broiler meat samples were too low to detect by direct plating.
In conclusion, the MRSA concentrations in the broiler meat samples were too low to be detected with the spatula method. Furthermore, we could confirm, that the numbers of MRSA in broiler meat are very low.

A17

Induction of resistance to Roundup, a glyphosate-containing herbicide, in Enterobacteriaceae in vitro

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Keywords: glyphosate, Enterobacteriaceae, resistance

Background and objectives: Roundup LB plus (RU) is the most common glyphosate-containing herbicide in Germany. Antimicrobial effects of glyphosate have recently been recognised. The objective of this study was to investigate the ability of RU to induce resistance in Enterobacteriaceae of animal origin as well as cross-resistance to antibiotics and fitness costs in the resulting resistant mutants.
Materials and methods: Ten isolates each of *Escherichia coli* and *Salmonella enterica* serovars from pigs and cattle were passaged daily at increasing concentrations of RU. Stable resistant isolates and respective ancestors had their whole genomes sequenced, fitness costs assessed by growth curves and cross-resistance to antibiotics determined by Vitek.
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 (MIC) to RU. Mutations associated with glyphosate resistance were found in all *Salmonella* isolates but not in *E. coli*. There were no changes in antibiotic resistance profiles or fitness costs.
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 use may result in preferential selection of pathogenic *Salmonella* bacteria that can persist in the environment.

A18

The occurrence of Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-E), *Clostridium* spp. and *C. difficile* in zoological gardens in Germany and Israel

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Keywords: Clostridium spp.*, Clostridioides difficile, ESBL-enterobacteriaceae*, *exotic animals, prevalence*

Background and objectives: In humans, ESBL-E are considered as a marker for multi-drug resistance, associated with increased illness severity, length of hospital stay and costs. *C. difficile* is the main pathogen for hospital-associated diarrhoea. We aimed to perform a prospective study, investigating these bacteria in exotic animals in zoological gardens.

Materials and methods: Fecal samples were collected in one zoological garden in Germany (125 samples) and two zoological gardens in Israel (105 samples). Samples were enriched and sub-cultured. Bacterial species were identified via Vitek-2 (ESBL-E) and MALDI-TOF (*Clostridium* spp). *C. difficile* strains were analyzed by capillary gel electrophoresis based PCR ribotyping.

Results: In Berlin**,** carriage rate of ESBL-E was 22% (27/125), including mainly *E.coli* isolates (96%). *Clostridium* spp. were detected in 4% of animals (5/125). *C. difficile* was detected in a Mhorr gazelle; PCR-ribotyping proved ribotype 126 which was moxifloxacin-resistant and genetically related to the human-hypervirulent ribotype 078.

In Israel, ESBL-E were detected in 28% (29/105) of animals, mainly *E.coli* isolates (83%). These results were not statistically different from the german zoological garden. Analysis for *Clostridium* spp. is currently in process.

Conclusions: The results showed highly related plasmids from different reservoirs. This indicates a possible plasmid-mediated spread and zoonotic potential of *bla*$_{CMY-2}$ carrying plasmids across the *E. coli* host populations.

A19

Non-specific hygiene- and management interventions do not reduce broiler colonization with ESBL-/ AmpC- producing *E. coli*

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Keywords: ESBL, colonisation, broiler

Background and objectives: The colonisation of broilers with ESBL- and AmpC- producing Enterobacteriaceae is well known and close contact to broiler flocks or through contaminated retail meat could lead to the transfer to humans. We used a newly established broiler colonisation model to investigate potential intervention strategies regarding hygiene- and management measures to reduce colonisation of broilers with these resistant bacteria.
Materials and methods: Groups of 90 broilers were housed in conventionally, alternating one measure each. Alternative parameters included acidification of water, alternative breed, reduction of stocking density and increased amount of litter. One fifth of the ESBL-/ AmpC- negative day- old broilers were orally co-infected on their third day of life (seeders) with 10^2 cfu of one ESBL- and one AmpC- producing *E. coli* strain. Colonisation success of all infected broilers (seeder, n=18) and 28 non- infected broilers (sentinel) was proven by cloacal swabs over the period of the trial and a final section.
Results: None of the so far studied parameters is able to significantly reduce the colonisation of broilers with ESBL-/ AmpC- producing *E. coli*. Water acidification seems to increase colonisation.
Conclusion: Apparently, none of the non-specific interventions are a meaningful approach for a reduction of ESBL-/ AmpC- producing Enterobacteriaceae in conventional broiler farms. More specific measures are therefore to be tested in future.

A20

Development of an Antibiotic Stewardship (ABS) Program for urinary tract infections and pyoderma in small animal medicine

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Keywords: multidrug resistance, companion animals, antibiotic stewardship

Background and objectives: The spread of antibiotic-resistant bacteria is currently challenging physicians in both human and veterinary medicine. However, primary preventive approaches such as interdisciplinary veterinary Antibiotic Stewardship (ABS) concepts are not available in Germany. For setting up local therapeutic recommendations, we prospectively investigate samples from patients with two relevant clinical diseases: urinary tract infection (UTI) and pyoderma.
Materials and methods: In total, 350 clinical samples (urine collected by cystocentesis or catheterisation and skin swabs) for each of the two indications will be obtained from dogs and cats and analysed using microbiological diagnostics including VITEK2 antimicrobial susceptibility testing and screening for specific resistance phenotypes.
Results: So far, 42 samples were analysed per indication. In 60% of the investigated urine samples bacteria were detected. The most prevalent species was *Escherichia coli* (17/25 samples). Among these, 24% showed a non-susceptible phenotype for amoxicillin, trimethoprim-sulfonamides and amoxicillin-clavulanate, which are often recommended as first-line antibiotics. In skin swabs, the most prevalent species were staphylococci (36/42 samples) including 5 expressing methicillin-resistance (1 *Staphylococcus aureus* and 4 *Staphylococcus* sp. of the *intermedius* group).

Conclusion: Ongoing analyses will provide more detailed information to develop ABS concepts for German veterinary clinics.

A21

Risk factors for the occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) in dairy herds - an update

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Keywords: Risk factors, MRSA, dairy cows

Background and objectives: Livestock associated MRSA have been found in dairy herds worldwide. Consequently, dairy cows could act as a reservoir of infection for other farm animals and for the human population. The objective of this study is to summarize the risk factors for the occurrence of MRSA in dairy herds and to identify the respective knowledge gaps.
Material and Methods: Literature was systematically screened using the key words MRSA and dairy cows.
Results: A higher MRSA prevalence was shown for dairy farms with improper milking hygiene, as described for other contagious mastitis pathogens. There is some evidence that dairy farms harboring additional animal species, especially pigs, are more frequently affected by MRSA. Previously, higher numbers of infection were reported in conventional dairy farms than in organic farms and as well in farms with a larger herd size. Furthermore, coagulase negative *Staphylococci* (CNS) with resistance genes like the *mecA*-gen in milk have frequently been reported in affected dairy farms. Finally, the antimicrobial selection pressure may provide a source of new MRSA strains, but despite decades of dry cow therapy with β-lactam-antibiotics MRSA prevalence is still quite low in dairy cows.
Conclusion: The risk factors for the occurrence of MRSA in dairy herds are improper milking hygiene, contact with pig farms, a conventional production system, larger herd size and CNS harbouring resistance genes.

A22

Determination of biocide susceptibility: Development of a broth microdilution method

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Keywords: biocide, broth microdilution, Staphylococcus aureus

Background and objectives: Proper disinfection is crucial to reduce transfer and spread of zoonotic bacteria in human and veterinary medicine. Biocide susceptibility testing lacks standardized methods in contrast to biocide efficacy testing. Therefore, a broth microdilution method for biocide susceptibility testing was developed based on a broth macrodilution protocol.
Materials and methods: The *S. aureus* reference strain ATCC® 6538 was comparatively investigated seven times by broth microdilution for its susceptibility to benzalkonium chloride (BAC), glutardialdehyde (GLU) and chlorhexidine (CHX). The inoculum preparation was performed with two different cultures (1st SC, 2nd SC), using a direct colony suspension method (DCS) with and without glass beads (GB). The results were read after 24 h, 48 h and 72 h of incubation at 37°C.
Results: The most common minimal inhibitory concentration (MIC) values were 0.000125 % for BAC (n=51/84), 0.125 % for GLU (n=44/84) and 0.00006 % for CHX (n=59/84). The modal MIC ± one dilution step was defined as acceptable range. In total, 88-100 % of the values were within this range. Based on our results, following proposal is made: use of a fresh overnight culture (1st SC or 2nd SC), inoculum preparation via DCS with or without GB, and incubation at 37°C for 24 h

Conclusion: This method can contribute to a harmonization of the biocide susceptibility testing of bacterial pathogens in routine diagnostics.

A23

Decline of ESBL-producing *E. coli* in broiler manure under practical conditions

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Keywords: ESBL, chicken-manure, environment

Background and objectives: ESBL- producing *E. coli* are frequently found in broiler stables. Chicken-manure is used to fertilize arable land. We quantified these resistant bacteria in the manure to assess the risk of this transmission path to the environment.
Materials and methods: The manure heap of an ESBL-positive stable was piled up behind the barn and investigated by taking 57 superficial and deep manure-samples in total for five consecutive days. Temperature and dryness of the manure were monitored and both ESBL-producing and non-resistant *E. coli* were quantitatively and qualitatively detected. The total number of Enterococci was captured as gram-positive comparison.
Results: We found a mean of $1{,}16*10^7$ CFU/g ESBL-producing *E. coli*, $1{,}05*10^8$ CFU/g total *E. coli* and $2{,}72*10^7$ CFU/g Enterococci in the litter one week before the animals were housed out and the stable was cleansed. The number of ESBL-producing *E. coli* decreased continuously during litter storage and a quantification was possible until 72h after removing the litter for the deep and 96h for the superficial samples. A qualitative detection was impossible for 3 deep samples after 72 and 96h respectively. The number of Enterococci varied imperceptibly. The manure heated up to 52°C in the centre of the pile, despite ambient temperatures of up to -15°C
Conclusion: We observed a fast decline of ESBL-producing *E. coli* during litter storage in winter. We will reperform it in the summer to ascertain climatic influences.

A24

Animal- and food-related Staphylococcaceae: A reservoir for beta-lactam resistance determinants

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Keywords: coagulase-negative staphylococci, macrococci, methicillin resistance genes

Background and objectives: Besides the *mecA* and *mecC* genes found in MRSA, methicillin resistance genes have also been reported in coagulase-negative staphylococci (CoNS) and macrococci, thereby representing a putative reservoir for resistance determinants. This study aimed to characterize methicillin resistance among CoNS and macrococci isolated from food samples and animal sources.
Materials and methods: Porcine nasal swabs, pigsty and foodstuff samples were screened for CoNS and macrococci. Strains were identified using MALDI-TOF mass spectrometry and 16S rRNA gene sequencing and resistances were determined by VITEK 2. Beta-lactam resistant strains were tested for the presence of *mecA*, *mecB*, *mecC* and *mecD* by PCR.
Results: Overall, 113 isolates were recovered including *S. sciuri* (n = 41), *S. haemolyticus* (n = 36), *S. cohnii* (n = 20), *M. caseolyticus* (n = 14) and *S. epidermidis* and *S. hominis* (each n = 1). While all *M. caseolyticus* isolates were susceptible to oxacillin, 95 CoNS isolates were tested resistant against oxacillin with MIC values $\geq$ 0.5µg/ml. Most oxacillin-resistant isolates (n = 90) carried the *mecA* gene, followed by 5 isolates carrying both, *mecA* and *mecC*. Interestingly, in one phenotypically oxacillin-resistant isolate, no *mec* genes were found. Two isolates tested phenotypically susceptible were also found to carry *mecA*.
Conclusion: The methicillin resistance genes *mecA* and *mecC* were frequently found in animal- and food-related CoNS highlighting the potential for these species to act as a reservoir for methicillin resistance genes.

Poster Session Novel Methods, Diagnostics and NGS

D01

Evaluation of B1 gene and G529 repeated element as PCR targets for diagnosis of toxoplasmosis in pregnant and aborted women in Erbil

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Keywords: Toxoplasmosis, B1 gene, G529

Background and objectives: Toxoplasmosis is a ubiquitous parasitic infection, transmitted vertically to the foetus causing undesired pregnancy outcome. This study aimed to evaluate PCR, targeting B1 gene and G529 comparing with ELISA. The study also aimed to assess placenta versus blood specimens as source of parasite DNA.
Methods: 350 women who attended Maternity Teaching Hospital in Erbil, were enrolled in the study and included pregnant women with and without history of abortion, and women at labour. Toxoplasmosis detected by IgG and IgM ELISA, as well as by PCR targeting B1 gene and G529. The period of the study was 10 months from November 2015 to September 2016.
Results: Of 350 samples, ELISA-IgM and ELISA-IgG were positive in 38(10.9%) and 81(23.1%), respectively. 7(2%) samples revealed seropositive for both IgG and IgM. PCR, was positive in 92 (26.3%) and 41 (11.7%), targeting B1 gene and G529, respectively. In 40 placenta samples, 1 (5.56 %) and 5 (27.77 %) of abortive women, versus 1 (4.55 %) and 5 (22.73 %) of non-abortive women revealed positive tests for B1 and G529, respectively and no significant association of toxoplasmosis and abortion was observed. While in 40 blood specimens tested by PCR, 7(38.88%) and 2(11.12%) abortive women revealed positive reactions by B1 and G529, respectively, versus 9 (40.90 %) and 4 (18.18%) of non-abortive.
Conclusion: PCR along with serology is insisted demand for definitive diagnosis of toxoplasmosis, and G529 being more efficient than B1 gene to be used in PCR.

D02

Part of the Outbreak or Not? – Investigation of a feline cowpox-outbreak

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Keywords: WGS, CPXV, outbreak

Background and objectives: In 2015, cowpox virus (CPXV) infections were observed in 5 cats treated in a small animal clinic in Hannover, Germany. As four out of five hospitalized cats showed identical hemagglutinin (HA) gene sequences, a transmission within the clinic was suspected.
Materials and methods: Whole genome sequencing (WGS) was conducted on 16 samples including 9 CPXV reference strains. The outbreak-associated viral DNA was extracted from cell culture of fresh feline tissues as well as from paraffin-embedded specimens. For phylogenetic analyses a MAFFT-alignment was generated. A distance matrix based on concatenated SNPs was calculated and plotted as dendrogram using Unweighted Pair Group Method with Arithmetic mean (UPGMA).
Results: Aligning of about 200.000 nucleotides of genomic sequences from 4 cases revealed 3 identical sequences and one genome that differed in 65 nucleotides. Genomes obtained from viable virus isolates and from paraffin-embedded lesion materials were identical.
Conclusion: Although identical HA gene sequences had been initially obtained from four hospitalized cats, genomic sequencing proved that a hospital-related transmission had occurred in only three cats. This study confirmed the coexistence of different CPXV clones in confined natural habitats. Thus, analyzing the rather short sequence of the hemagglutinin gene is not sufficient to investigate CPXV outbreaks nowadays. Moreover, superior WGS method can be applied to paraffin-embedded specimens.

D03

Detection of MAP in faeces and milk samples by LAMP

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Keywords: MAP, LAMP, Mycobacterium avium ssp. *paratuberculosis, Crohn´s disease*

Paratuberculosis is a disease of ruminants which is becoming increasingly important in countries with strong dairy industry as it leads to a declining milk production and higher replacement rates. Additionally *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is associated with Crohn's disease as a potential zoonotic infection. Consuming raw milk or products thereof poses a potential health risk to the consumer.
In Germany, paratuberculosis is listed as a notifiable animal disease. Since November 2017, there is an official control program to combat MAP in Lower Saxony which includes sampling of blood, faeces and milk and subsequent analysis for antibodies or the pathogen itself. However, isolation and cultivation is challenging.
To improve, simplify and accelerate the current detection method via PCR, a novel detection method via loop mediated isothermal amplification (LAMP) was developed. LAMP is said to be faster and more sensitive compared to PCR. Moreover, it is less vulnerable to disruptive factors, which makes LAMP a good choice for direct testing of sample material.
So far, the development of the assay is complete and it is currently being tested on various samples of ruminants (milk and faeces).
The LAMP method is a valuable diagnostic tool for the detection of MAP and can be an alternative to PCR testing. The findings of the study can also be helpful to develop detection methods for pathogens of the *Mycobacterium tuberculosis* complex.

D04

Multiplex screening approach for rapid STEC diagnostic

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Keywords: E. coli, food, real-time PCR

Background and objectives: In 2015, 6025 human STEC infections were reported. O157 is still the most prevalent serogroup. Therefore, many member state surveillance and industry own-control programmes set their focus on the diagnostic of this serotype. Addressing this needs, we developed a rapid real-time PCR based system for the simultaneous detection of the major STEC associated virulence factors Stx1, Stx2, intimin and serogroup O157.
Materials and methods: Based on TaqMan® technology a 4plex real-time PCR system (SureFast® STEC 4plex) was created. The test was subjected to inhouse validation evaluating specificity, ruggedness, accuracy, LOD, and stability. Technical validation was performed on 8 different devices. A sensitivity study was performed by spiking 15 food matrices from 5 categories. DNA extraction was performed by both a spin filter and a newly developed rapid method.
Results: Specificity of the assay was successfully determined by testing *E. coli* strains belonging to O157 and other serogroups (e.g. O26, O111, O145) harbouring virulence determinants *stx1a-d*, *stx2a-g* and *eae*. Differentiation of the single targets was performed in FAM: *stx1* and *stx2* variants, ROX: O157, Cy5: *eae*, and VIC: IAC. Exclusivity study was correctly run with strains from genera closely related and/or commonly detected in food.
Conclusion: This diagnostic test allows the rapid and sensitive detection of the most important STEC associated virulence factors and the main important serotype O157 for an efficient monitoring in the food and feed industry.

D05

Comparison of three molecular methods for detection of *Toxoplasma gondii* in pork

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Keywords: toxoplasmosis, food, PCR

Background and objectives: Comparison of epidemiological data on the occurrence of *Toxoplasma gondii* tissue cysts in meat is hampered by the lack of standardization and a great variety of methods for molecular detection. This study aimed to compare and validate three different PCR methods for detection of *T. gondii* DNA in pork.
Materials and methods: Analytical performance characteristics of two qPCRs (Tg-qPCR1, Tg-qPCR2) and one conventional PCR, all targeting a 529 repeated element, were assessed using genomic *T. gondii* DNA of the three main genotypes.
Results: qPCR efficiencies for all three genotypes ranged between 93.8-94.4% (Tg-qPCR1) and 94.3-95.6% (Tg-qPCR2). Tg-qPCR2 displayed a lower 95% detection limit (LOD) of 1 GE/PCR compared to Tg-qPCR1 with a 95% detection limit of 10 GE/PCR. However, both qPCR assays could detect *T. gondii* DNA at concentrations as low as 0.1 genome equivalents (GE)/PCR and showed an overall PCR performance score of 85%. Reliable quantification is possible over 4 log ranges from 10^5 to 100 GE/PCR with a mean repeatability relative standard deviation of ≤11% and a reproducibility relative standard deviation of ≤23.3%. The conventional PCR proved to be highly sensitive (LOD 100 fg), but not suitable for detection of *T. gondii* DNA in pork as unspecific amplification of porcine DNA was observed.
Conclusion: In contrast to the conventional PCR, the two studied qPCRs are similarly suitable for sensitive and specific detection of *T. gondii* DNA in pork.

D06

Antimicrobial resistance and population structure of *Pseudomonas aeruginosa* strains from animals

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Keywords: Pseudomonas, resistance, animal

Pseudomonas (*P.*) *aeruginosa* is a leading cause of hospital-acquired infections worldwide. It poses a therapeutic challenge because of the emergence of multidrug resistant strains, i.e. 3/4MRGN isolates that show resistance to 3 or 4 of the clinically relevant drug classes (ureidopenicillins, 3rd/4th-gen. cephalosporins, carbapenems, and fluoroquinolones). We examined *P. aeruginosa* from animals for resistance genomic features to unravel putative overlaps with human isolates.

From 2014-2018 211 *P. aeruginosa* strains were isolated from various clinical sites from cats/dogs (74.4%), livestock (6.2%), and other animals (19.4%). Antimicrobial susceptibility was tested (VITEK 2) and MICs were interpreted according to CLSI. Strains were screened for carbapenemase genes and multilocus sequence types using PCR and NGS.

Thirty (14.2%) strains were classified as 3/4MRGN; 11.4% of the strains were imipenem resistant. While acquired carbapenemases were not detected, variations in the OprD sequence correlated well with the strain´s resistance to imipenem. The finding of ST244 and ST395 – international high-risk clones that are frequently associated with human infections – together with the finding of 3/4 MRGN in animals suggests that *P. aeruginosa* strains might be transmitted between humans and animals. Detailed genomic and functional comparative analyses are necessary to further clarify the impact of animal *P. aeruginosa* strains for public health.

D07

Metagenomics: virus-host ratio as a crucial determinant for identifying zoonotic pathogens

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Keywords: NGS, Metagenomics, orthobornavirus

Background and objectives: Retrospective analyses of human encephalitis cases using next generation sequencing metagenomics approaches enabled the identification of novel or unexpected zoonotic viruses, like the novel variegated squirrel bornavirus 1 and the classical borna disease virus 1 (BoDV-1). However, several factors determine the sensitivity of metagenomic analyses in diagnostic procedures; a major determinant is the dataset size.
Materials and methods: We performed rarefaction analyses with several datasets, which originated from BoDV-1 positive human encephalitis cases from Germany, to determine the necessary size of datasets to detect this pathogen with a certain probability. To this end, we randomly subsampled the datasets and mapped the reads along the BoDV-1 reference sequence to analyze the number and proportion of virus reads and contigs, respectively.
Results: In an example with high virus load (Cq 19-20), we detected 5-10 reads in subsamples of 10.000 reads. This shows that the virus/host ratio strongly affects the output of viral reads in datasets. While the lengths of the generated contigs, i.e. the coverage of the genome, reached a plateau very fast, there was a proportional increase of sequence depths and subsample sizes. In one case, we observed an over-representation of the 3' prime end of the BoDV-1 genome, which might reflect the transcription gradient of BoDV-1 during replication.
Conclusion: The results will help assess the reliability of metagenomic results.

D08

Luminex-based serological determination of *Toxoplasma-gondii* infection in chickens

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Keywords: Toxoplasma-Luminex-bead assay, Chickens, Multiplexing serological assays

Background and Objective: The detection of *Toxoplasma gondii* in animals relies primarily on serological assays. Here, we aimed to establish a Luminex-bead assay for the specific and sensitive detection of *T. gondii* infections in chickens. Because Luminex-bead assays enable the simultaneous analysis of multiple analytes within a single biological sample this assay may represent one of the components of future multiplex assays for avian serological monitoring.
Materials and Methods: Commercially available magnetic Luminex-beads were coupled with streptavidin, to which recombinant biotinylated *T. gondii* surface antigen TgSAG1 was attached. Chicken serum antibodies binding to TgSAG1 were detected by a fluorophore-coupled secondary antibody. Other Luminex-beads to which anti-chicken IgY and chicken serum albumin had been coupled served as positive and negative control, respectively. The assay was validated with sera from naturally and experimentally infected chickens.
Results: Chickens from which *T. gondii* had been isolated by mouse bioassay showed high fluorescence-intensity values. The examination of sera from chickens without *T. gondii* isolation and seronegative in reference tests (immunofluorescence assay, modified agglutination assay) revealed only background reactions in the Luminex-TgSAG1-Assay.
Conclusion: The Luminex-TgSAG1-Assay seems to represent a suitable method for the detection of *T. gondii* infections in chickens.

D09

Isothermal nucleic acid amplification as a versatile tool for pathogen detection and monitoring

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Keywords: Isothermal amplification, POC, rapid test

Background and objectives: The use of nucleic acid amplification techniques has significantly improved the specificity and sensitivity of diagnostic tests for the detection of pathogenic organisms. However, the PCR usually remains laboratory-bound and is not available for on-site tests. Alternatively, isothermal nucleic acid amplification can be utilized.
Materials and methods: In the past, several assays and systems for the detection and characterization of numerous pathogenic organisms based on isothermal nucleic acid amplification have been established in our group. Alternative read-out options included naked-eye (turbidity and colorimetry), spectrometric, fluorometric and lateral-flow dipstick formats were tested and optimized to offer maximum flexibility for system integration.
Results: We were able to demonstrate the specific and highly sensitive detection of pathogens in less than 20 minutes. A special primer/probe – system allows the direct detection of the amplification product on lateral-flow-strips. The reaction can work instrument-free and the result is given in a format which can be read out by the untrained eye. Further application-oriented systems include assays for microfluidic lab-on-chip devices and the usage in rudimentary diagnostic equipment.
Conclusion: The flexibility of isothermal nucleic amplification offers new possibilities for point-of-care tests and may complement existing methods and technologies as PCR and immunochromatographic on-site tests.

D10

Tick activity at day and night: Development of a Trail Camera and software assisted system for tick activity monitoring

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Keywords: tick, activity, night

Only little is known about the diurnal questing activity of ticks, especially *Ixodes ricinus.* We describe the development of a Trail Camera and software assisted system to monitor tick host seeking activity during night and day at a very narrow time frame.
We used field-plots of 0,5m² size with tops covered by heating cable to prevent the ticks from leaving the plot. Ticks were supplied with PVC rods of 2 mm thickness and 20 mm width for questing. Two cameras were placed in such way that each camera took pictures of one of the wider sides of the plc. rods. Pictures were taken every 3 Minutes and active questing ticks were counted by a software. During night low glow infrared LEDs were used as flash to take pictures.
Evaluation of the pictures taken showed continuing activity of *Ixodes ricinus* during night hours. Ticks kept on questing during night and although some ticks returned to the ground others became active.

D11

Serological diagnosis of zoonoses and production diseases in pig herds using a protein microarray

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Keywords: pig, serology, microarray

The national salmonella monitoring in Germany already provides valuable food safety information on *Salmonella spp.* in fattening pigs at herd level. However, there is a high demand for a screening test for multiple zoonotic agents like *Yersinia enterocolitica, Toxoplasma gondii* or *Trichinella spp.* as they belong to the most relevant biological hazards in the context of meat inspection of swine (EFSA 2011). In order to realize a broad serological monitoring of these pathogens, a cost-efficient diagnostic method suitable for routine testing has been missing so far.
For this reason, a protein microarray chip was developed for the detection of antibodies against several zoonotic agents, as well as for pathogens causing production diseases in pigs. The screening test can be conducted simultaneously to the established salmonella monitoring as it is based on the same sampling material.
By now, it is possible to detect specific antibodies against *Salmonella spp.*, *Yersinia enterocolitica*, *Toxoplasma gondii*, Hepatitis E virus, Influenza A virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and PRRS virus via microarray analysis. The test system can be implemented as a mono-cup format or as a 96-well format which allows high sample throughput. The serological data at herd level can be used to improve food chain information and provides a valuable tool to continuously optimize herd health.

D12

Whole genome sequencing of *Listeria monocytogenes* food isolates reveals population structure and facilitates outbreak clarification

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Keywords: Listeria monocytogenes, molecular typing, WGS

Background and objectives: Listeriosis is a foodborne infectious disease, caused by the zoonotic pathogen *Listeria monocytogenes* (*Lm*). Although comparatively rare, a steadily increasing number of human cases together with a high case fatality rate render the disease a major public health concern. For effective surveillance and disease control, comprehensive molecular typing of *Lm* isolated from food, food-processing plants and humans is indispensable. Still, the analytical basis needs to be improved and validated.

Materials and methods: *Lm* isolates from ready-to-eat food and food-processing plants sampled by official food inspectors in Germany in 2016 were analysed using pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS). For WGS-data interpretation, different analytical approaches were tested and compared.

Results: PFGE and WGS-based typing divided *Lm* isolates into various clusters, but resolution differed markedly between methods and analytical approaches. While *Lm* isolates from meat products displayed a high level of diversity, isolates from fish products were less diverse, with various closely related strains showing supra-regional distribution. Furthermore, isolates from meat products more frequently revealed MLST clonal complexes known to be associated with human infections.

Conclusion: Representative typing data elucidate the population structure of *Lm* isolates and allow tracing of human cases to contaminated foodstuffs.

D13

Exploring "big sequence data": De-novo detection of viral pathogens by a dynamic database approach

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Keywords: Next-generation sequencing, pathogen detection, big data analysis

Background and objectives: Emerging and re-emerging viral infectious diseases cause frequent threats to both human and animal health. The lack of diagnostic methods for these novel pathogens can lead to severe delays in detection and subsequently counteracting the disease. Diagnostic sequencing by unbiased next-generation sequencing is a key method for the detection and identification of pathogens and also allows the characterization of mixed infections. The amount of data in such metagenomics-oriented sequencing approaches is continuously growing, thus impeding comprehensive exploitation of the entire data. The interdisciplinary project DetektiVir developed a new approach by combining molecular nucleic acid-based virus detection by sequencing and customized serological diagnostics in a dynamic database.
Results: Central part is a novel diagnostic data hub that combines raw sequence reads and metadata with the results from taxonomic classification software in a low-code platform. The core system offers flexible interfaces and algorithms in a user-friendly environment, enabling analyses and evaluation of big sequencing data across different samples. While implementing, we were already able to discover novel viruses like an ovine picornavirus and a new paramyxovirus with putative zoonotic potential.
Conclusion: Exploring metagenomic sequencing data is an ongoing challenge. Using a dynamic database approach we were able to pave the way to earlier identification of pathogens.

D14

Establish differentiated airway epithelium models from harbor seals to study the course of infection by different pathogens

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Keywords: Harbor seal, Differentiated airway epithelium

Background and objectives: For decades, massive infections by viral and bacterial pathogens in harbor seals (hs) have been reported. These pathogens included influenza viruses, distemper viruses, herpesviruses, *Streptococcus spp.* and *Bordetella spp*. Lack of a suitable cell culture system comprising polarized hs airway epithelial cells limits the investigation of how pathogens infect or invade the airway.
Materials and methods: To study the effects of pathogen infection in the harbor seal airway epithelium, we applied an *ex vivo* precision-cut lung slices (PCLS) technique, and *in vitro* primary trachea epithelial cell cultures (PTEC) to analyze pathogen-induced effects on the respiratory epithelium
Results: PCLS contain airway cells in the original setting including ciliated cells, goblet cells, basal cells and sub-epithelial cells. This culture system is suitable to study the cell tropism of the above-mentioned pathogens. PTEC of harbor seals could be maintained for three passages. They express sialic acids on the cell surface which is consistent with the high susceptibility of harbor seals to infection by influenza viruses. Furthermore, we established an air-liquid interface culture system for well-differentiated harbor seal tracheal epithelial cells (hsALI) which contains ciliated and mucus producing cells.
Conclusion: These culture systems will be invaluable tools to study the entry route and the cell tropism of microbial respiratory pathogens of marine mammals.

D15

Molecular detection of Puumala orthohantavirus: struggling with high nucleotide sequence variability

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Keywords: Puumala orthohantavirus, bank vole, sequence types

Puumala orthohantavirus (PUUV), family *Hantaviridae* is the most common causative agent of haemorrhagic fever with renal syndrome (HFRS) in Europe. PUUV is a negative-stranded RNA virus with a trisegmented genome. The S-segment encodes two open reading frames (ORF) for the nucleocapsid (N) protein and a non-structural (NSs) protein. To date, eight genetic lineages of PUUV have been described, all associated with the bank vole (*Myodes glareolus*) as reservoir.

Our investigations by conventional RT-PCR in Germany showed that PUUV of the Central European lineage is associated with the presence of the Western bank vole phylogroup. Human PUUV infections occur only in western and southern areas of Germany. In contrast, PUUV-infected bank voles in Poland belong to the Carpathian and Eastern evolutionary lineages. Bank voles collected in Germany during 2010-2013 revealed the occurrence of PUUV sequence types of the N- and NSs-ORF with temporal and/or spatial variation. The nucleotide sequence divergence within the S segment of PUUV strains reached 20% causing problems to select primer and probe sequences for PUUV real-time RT-PCR.

Currently, we are evaluating several real-time RT-PCR assays for detection of PUUV strains of different origin. Perspectively, we intend to design a diagnostic platform allowing comprehensive and

timesaving molecular bed-side and pen-side diagnostics of PUUV and pathogens causing similar symptoms.

Poster Session New and Re-Emerging Zoonotic Diseases

N01

Productive Propagation of Rift Valley Fever Vaccine Strain MP-12 in *Rousettus aegyptiacus* Fruit Bats Confirms this Species as a Putative Reservoir Host

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Keywords: Rift Valley fever virus MP-12 vaccine strain; virus reservoir; Rousettus aegyptiacus bat

Background and objectives: Rift Valley fever Phlebovirus (RVFV) can infect a variety of species, predominantly ruminants, camelidae and humans, and RVFV infection may lead to serious, possibly fatal disease in human. Although the main reservoir of the virus is not yet identified, small mammals such as rodents and bats may act as amplifying hosts.
Materials and methods: We therefore immunized *Rousettus aegyptiacus* fruit bats that are abundant in Northern Africa with the vaccine strain MP-12, in order to elucidate the competence of this species for virus propagation and transmission.
Results: We were able to detect the RVFV genome in the spleen of each of the immunized animals, and we re-isolated virus from the spleen and liver of some animals. Moreover, we were able to identify the Gc RVFV surface antigen in mild subacute multifocal necrotizing hepatic lesions of one bat that had been sacrificed 7 days post immunization.
Conclusion: These findings prove the competence of this species to propagate RVFV.

N02

Development and evaluation of an in vitro skin infection model for the zoophilic dermatophyte *Trichophyton benhamiae*

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Keywords: emerging zoonotic pathogen, Trichophyton benhamiae, 3D infection model

Background and objectives: The zoophilic dermatophyte *Trichophyton (T.) benhamiae* causes highly inflammatory dermatophytoses in guinea pigs (GP). Through close contact, it is also transmissible to humans evoking the same clinical presentation which often requires difficult and long lasting treatments. Previous *in vivo* and *in vitro* studies revealed diverging expression patterns of specific virulence factors. Hence, the need for an infection model more closely resembling the *in vivo* situation is inevitable.
Materials and methods: Optimal guinea pig skin explant (GPSE) culture conditions for infection studies were determined. Skin morphology was examined histologically and immunohistochemically (HE, anti-Ki67, TUNEL). Dermatophyte strains isolated from GP and humans were cultured to produce highly concentrated solutions of infectious units. After application to GPSE, infection process and expression of virulence factors were monitored (PAS-reaction, immunofluorescence stainings with antibodies against *T. benhamiae* and virulence factors).
Results: The histological evaluation confirmed a maintained integrity of GPSE during culture. Infection experiments were conducted successfully with defined doses of infectious units of *T. benhamiae* strains. The examined virulence factors were detected at protein level.
Conclusion: A highly suitable 3D infection model based on GPSE was established to elucidate the pathomechanisms of *T. benhamiae* during skin infection.

N03

First phylogenetic analyses of TBE virus detected in Lower Saxony

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Keywords: Tick-borne encephalitis, non-endemic region, Lower Saxony

Background and objectives: Tick-borne encephalitis (TBE) is the most important arboviral disease in Europe. In Germany the main endemic regions are located in the south. However, sporadic human TBE cases are reported also outside of the known endemic areas. The knowledge of invading TBE virus strains is of importance for a better surveillance of this important disease.
Materials and methods: Ticks were sampled in 2018 in several locations of Lower Saxony associated with human cases and/or human sero-positivity. Ticks were pooled according to stage and sex. Testing for TBE viral RNA was done using the RT-qPCR (Schwaiger & Cassinotti 2003). In positive pools the E gene was amplified and sequenced for phylogenetic analysis.
Results: A total of 1031 ticks were sampled and tested. Two positive pools could be detected in the areas of "Rauher Busch" and "Barsinghausen/Mooshütte". The whole E genes (1488 nucleotides) of the two TBE virus strains could be amplified and analysed. According to these data, the two virus strains are closely related to each other and cluster genetically with a TBE virus from Poland isolated in 1971.
Conclusion: This study provide for the first time data on the phylogeny of TBE virus in Federal State of Lower Saxony. The phylogenetic data imply that closely related TBE viruses are circulating in the two locations and that the origin of the TBE virus strains may originate from Poland. These results strengthen the hypothesis of an east-west invasion of TBE virus.

N04

Pathogen screening of rats from four breeding colonies in Germany

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Keywords: rats, breeding colony, pathogens

Rats can carry various pathogens with zoonotic or unknown zoonotic potential. Zoonotic pathogens are *Leptospira* spp., *Streptobacillus moniliformis* and possibly Rat hepatitis E virus (ratHEV) and *Acinetobacter baumannii*. Rat polyomavirus 1(RnorPyV1) and rat hepacivirus are most likely non-zoonotic, rat-specific agents.

In a pilot study, 81 Norway rats (*Rattus norvegicus*) and 65 Black rats (*Rattus rattus*) from four breeding colonies in Germany were examined by pathogen isolation approach, pathogen-specific PCR/RT-PCR methods and by multiplex serology.

Acinetobacter baumannii was isolated from tracheal samples in 11 of 127 analyzed rats (8.7%). In none of the 146 investigated rats, *Leptospira*-DNA was found. With a prevalence of 14.2%, eighteen of 127 samples were positive for ratHEV-RNA. *Streptobacillus* spp. were detected by PCR in 16 Black and 10 Norway rats of 127 analyzed rats (totally 20.5%). A few rat hepacivirus-positive animals (3/127; 2.4%) were found and in 50% (36/72) of the rats RnorPyV1-DNA was be detected. Multiplex serology analysis showed the presence of antibodies reactive to rat parvoviruses, murine pneumonia virus, rat rotavirus, *Streptobacillus* spp. and *Mycoplasma pulmonis*.

In conclusion, the results indicate a large number of different pathogens in both rat species within breeding colonies in Germany. This could result in new health and safety measures for the animal keepers.

N05

Pathogenesis of the novel highly pathogenic avian influenza H5N8 2016, H5N5 2017 and H5N6 2017 viruses in ferrets

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Keywords: HPAIV, ferret model, virulence

Background and objectives: The incursion of new reassortants of HPAIV H5Nx clade 2.3.4.4b resulted in epidemic outbreaks among wild birds and poultry throughout Europe. In this study, the potential of cross-species infection was evaluated in the main model for human influenza: the ferret.
Materials and methods: The pathogenicity of A/tufted duck/Germany/AR8444/2016 H5N8, A/turkey/Germany-SH/R425/ 2017 H5N5 and A/common_pochard/Germany-BY/AR09-18-L02421/ 2017 H5N6 was assessed by experimental i.n. inoculation of ferrets with 10^6 $TCID_{50}$/animal. Viral titers in nasal washes or organ samples were determined. Sero-responses were measured by ELISA (NP and H5 proteins) and HI assay.
Results: In general ferrets did not show respiratory symptoms or influenza related changes in body weight and body temperature. Interestingly one animal (out of five) that was inoculated using H5N5 isolate did shed virus in the nasal washing and exhibited viral genome load in several organs including the brain. All ferrets seroconverted.
Conclusion: Clade 2.3.4.4b H5 viruses revealed in general a mildly virulent phenotype in ferrets, consistent with lack of reported human cases. However, the potential of systemic spread of H5N5 in particular was demonstrated in the human model species.

N06

Vector competence of German *Aedes japonicus japonicus* for Zika virus

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Keywords: Zika Virus, Aedes japonicus japonicus, vector competence

Background and objectives: *Aedes japonicus japonicus* (*Ae. japonicus*) is widely distributed in Central Europe and currently established in at least 10 countries, including large parts of Germany [ECDC 2017]. Therefore we evaluated the vector competence of *Ae. japonicus* for ZIKV under representative temperature conditions for temperate regions.
Materials and methods: Field caught German *Ae. japonicus* were orally infected with Zika virus (ZIKV; strain ZIKV_FB-GWUH-2016, GenBank KU870645, fifth passage) at different temperatures (21°C, 24°C, 27°C). Female mosquitoes were analysed for infection, dissemination and transmission 14 days post infection (dpi). Infection and dissemination was determined by analysing bodies or single legs of mosquitoes for viral RNA using Real Star Zika Virus RT-PCR Kit (Altona diagnostics, Hamburg, Germany). Saliva was tested for infectious virus particles using the salivation assay as previously described [Heitmann et al. 2017].
Results: *Ae. japonicus* was susceptible to ZIKV infection at all temperatures, but dissemination was only observed for 24°C and 27°C and transmission was exclusively detected at 27°C (transmission rate: 14.3%).
Conclusion: *Ae. japonicus* is able to transmit ZIKV. The current risk is limited by the species" restricted distribution to Central Europe with temperate climatic conditions.

N07

Prevalence of Tula orthohantavirus and *Leptospira* spp. in common voles

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Zoonotic pathogens that can cause severe diseases in humans are on the rise worldwide. Rodents harbor several pathogens relevant for public health including species-specific pathogens in terms of rodent host species such as hantaviruses.

The common vole (*Microtus arvalis*) harbors a number of rodent-borne pathogens incl. Tula orthohantavirus (TULV) - a common vole-specific pathogen and *Leptospira* spp., a pathogen found not only in common voles, but also other vole and rodent species. This study aimed to determine the prevalence of TULV RNA and *Leptospira* spp. DNA in common voles and related *Microtus* species.

Common voles were snap trapped in the "Thüringer Becken", a central German region known for intensive large-scale agriculture where outbreaks of the common vole occur about every 2-5 years. Previously studies showed that both pathogens are generally present in common voles in this area.

We sampled 705 voles and analyzed lung samples for TULV using a standard S-segment-specific RT-PCR with subsequent sequence determination and kidney samples for *Leptospira* spp. using a lipl32 screening PCR followed by a secY-typing PCR.

First results show strong differences between pathogen prevalence in the common vole depending on the habitat Prevalences ranged between 0% and 58% for TULV and *Leptospira* prevalences ranged between 0% and 64% in *Microtus arvalis*. First hints suggest a positive association of the prevalence of *Leptospira* and the presence of water bodies.

N08

Strain Dependent Rift Valley Fever Virus Pathogenesis in Sheep

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Keywords: RVFV, viremia, sheep

Rift Valley Fever Virus (RVFV) is a zoonotic arbovirus of the order *Bunyavirale*s. Sheep are a susceptible host species for RVFV but depending on the age of the animals and on the infecting strain, clinical symptoms may vary.

In order to compare the RVFV pathogenesis we have challenged 6-month-old sheep with the RVFV strains 35/74 and ZH501 respectively. After four days half of the animals was necropsied all other animals after 20 days. We examined blood samples by virus re-isolation attempts (using 10fold dilutions) as well as in qRT-PCR. Additionally organ samples were screened by qRT-PCR.

Viremia was regularly detected at day two and three by re-isolation. Viral RNA could be detected as early as day two post infection (dpi) sporadically until the end of the trial. We found that liver and lung samples of day four post infection were highly positive but negative at day 20. Brain samples of our animals were all negative 20 dpi.

This infection study shows that these two RVFV strains have slightly different replication kinetics in sheep. Although all animals developed fever, clinical symptoms were rather mild and no animal needed to be euthanized for animal welfare reasons. Viremia titers in the blood of all animals challenged with both strains were sufficiently high to infect naïve susceptible mosquitoes that could then spread further the infection. All animals necropsied at day 20 could clear the systemic infection efficiently.

N09

Tick-borne pathogens in small mammals and ticks from Saxony - a reassessment of prevalence dynamics over the last decade

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Keywords: tick-borne pathogens, small mammals, ticks

Background and objectives: Ticks are important vectors for zoonotic pathogens. Rodents play an important role in the life cycle of ticks and as reservoirs for tick-borne pathogens (TBP). The aim of this study was to evaluate prevalence dynamics of TBP in rodents and ticks collected from habitats which were previously investigated. Material and methods: In the years from 2015-2017, 202 rodents were collected as well as 1,243 attached ticks and 577 questing ticks. DNA was extracted from small mammals' organs and from ticks. Samples were examined for the presence of *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Candidatus* Neoehrlichia mikurensis (CNM) and *Babesia* spp. via PCR.
Results: The prevalence in rodents was 46% for *B. burgdorferi* s.l., 23.7% for *Rickettsia* spp., and 55.9% for CNM. In attached ticks it was 3.3% for *B. burgdorferi*, 23.7% for *Rickettsia* spp., and 4.9% for CNM. Both, rodents and engorged ticks were negative for *Babesia* spp. The prevalence in questing ticks was 6.3% for *B. burgdorferi*, 18.1% for *Rickettsia* spp., 7.2% for CNM, and 1.4% for *Babesia* spp.
Conclusion: While the prevalence for *Babesia* spp. decreased in comparison to the years 2008-2014, the prevalence for CNM and *Borrelia* increased. Though prevalence levels differed, the pathogen species did not change (*R. helvetica*, *R. raoultii*, *Borrelia afzelii*, *Babesia venatorum*, *Ba. capreoli* and *Ba. microti*) over the years.

N10

Antiviral effect of the RNAi pathway on ZIKV infection in *Aedes aegypti*

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Keywords: RNAi, ZIKV, 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, but little is known for Zika virus (ZIKV). Understanding the interactions between viruses and the mosquito immune system is beneficial for understanding the development and dynamics of vector competence. This is especially relevant for viruses of public health concern, such as ZIKV.

dsRNA targeting key proteins of the piRNA and siRNA pathways are transfected into *Aedes aegypti*-derived Aag2 cells. Potential effects on subsequent ZIKV replication are determined. Similarly, dsRNA is injected into adult *Ae. aegypti* mosquitoes, which are subsequently infected with ZIKV via a blood meal and changes in virus replications are monitored.

In vitro knock-down of key proteins and their effect on ZIKV replication were determined. Interestingly, the knock-down of Ago2 was not sufficient to increase ZIKV replication, whereas Piwi4 knock-down caused an increase in viral titer. In vivo knock-downs in *Ae. aegypti* mosquitoes have been established for Ago2 and Piwi4.

The in vitro results indicate that ZIKV interacts with the siRNA and piRNA pathways of *Ae. aegypti* and has possibly evolved methods to evade it. In vivo experiments will elucidate whether this can have implications on the vector potential of the mosquito.

N11

Microtus spp. and other small mammal genera as reservoirs for bartonellae from Germany and the Czech Republic

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Keywords: voles, rodent-borne, Bartonellosis

Rodents are important reservoirs for zoonotic agents. Thus, the distribution of rodents and their vicinity to humans and companion animals may have an important impact on human and animal health. Bartonellae are bacteria which may cause cat scratch fever and other severe diseases. Most *Bartonella* spp. are carried by rodents. However the reservoir potential of some rodent genera, e.g. *Microtus*, has not yet been precisely examined in Central Europe. Therefore, we examined different small mammal species from Germany and the Czech Republic for *Bartonella*. Small mammals were collected during 2014-2016. DNA was extracted (Qiamp DNA Mini Kit, Qiagen) from spleens and examined for the presence of *Bartonella* DNA by conventional PCR targeting the *16S-23S rRNA* intergenic spacer region. In total, 321 animals were collected belonging to four genera: *Myodes* (n= 78), *Apodemus* (n= 56), *Microtus* (n= 149), *Sorex* (n= 38). *Bartonella* DNA was detected in 226 specimens (70.4%) from which 104/174 (59.7%) originated in Germany and 122/147 (83.0%) in the Czech Republic. The prevalence in each genus was: 47.4% (n= 37/78) for *Myodes,* 63.1% (n= 24/38) for *Sorex*, 82.5% (n= 123/149) for *Microtus* and 75%

(n= 42/56) for *Apodemus.* While the detected high prevalence for *Bartonella* in *Apodemus* and *Myodes* spp. is confirmatory with previous findings, the prevalence in *Microtus* spp. was unexpectedly high. This indicates that individuals belonging to this genus may also be potential reservoirs in Central Europe.

N12

Prevention of hantavirus-diseases and leptospirosis: experience of the public health authorities in Germany and expectations towards the RoBoPub consortium

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Keywords: zoonosis, qualitative research, risk mapping

Background and objectives: Hantavirus-disease and leptospirosis are rodent-borne diseases which are notifiable to the German local public health authorities (LPHA). LPHAs play an important role in primary and secondary prevention by informing the public about the disease risk and increasing the awareness among physicians. The RoBoPub consortium aims to support the LPHA with these tasks. We conducted a workshop to identify the needs of the LPHAs regarding health communication and expectations towards the RoBoPub consortium.
Materials and methods: The World Café method was used to elaborate the topics occupational and private exposure to rodent-borne diseases, and the use of risk-maps. Each café table was devoted to one topic and the discussion process was supported by key questions on flipcharts and a moderator. The 24 participants were split up into three groups, which rotated among the tables to discuss and contribute to each topic.
Results: Concerning occupational exposure, main result was the difficulty to reach the exposed groups like foreign seasonal workers. The greatest challenge for private exposure was the high proportion of unknown transmission routes. With respect to the risk maps, the spatial resolution and the mode of distribution of maps were major topics.
Conclusion: The World Café method was suitable to identify needs of LPHAs. These needs were communicated to the RoBoPub consortium to ensure their consideration.

N13

Amphibians and Reptiles as Reservoir Hosts for Rift Valley fever virus?

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Keywords: Rift Valley fever virus, reservoir host, amphibians and reptiles

Background and objectives: Rift Valley fever Phlebovirus (RVFV) is a mosquito-borne zoonosis, which has caused significant epidemics in Africa and the Arabian Peninsula. Infections can lead to serious clinical manifestations in ruminants and camels. Humans can develop a fatal hemorrhagic fever. Currently the main reservoir of the virus remains unknown, but a potential role of amphibians and reptiles in the virus ecology is assumed.
Materials and methods: To elucidate the potential of amphibians and reptiles to act as reservoir hosts for RVFV, 30 Agamas (*Agama agama*) and 30 toads (*Amietophrynus regularis*) were infected with a non-virulent RVFV MP-12 vaccine strain as well as the highly virulent ZH501 strain and clinical signs, virus shedding and amplification of virus as well as seroconversion were monitored throughout the experiment. After necropsy animal tissues underwent an immuno-/ histopathological investigation.
Results: RVFV MP-12 infections in Agamas as well as in toads were completely inconspicuous and even the highly pathogenic RVFV infections were imperceptible. However, RVFV ZH501 infection of *A. agama* led to amplification in the spleens of animals 3 days post infection and virus was shed occasionally to low extent. In general, seroconversion was observed with low significance in both species.
Conclusion: The results prove the potential role of reptiles to propagate and shed RVFV.

N14

First immunohistochemical characterization of target tissues of the Puumala orthohantavirus (PUUV) in its natural host - the bank vole (*Myodes glareolus*)

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Keywords: Puumala orthohantavirus, immunohistochemistry, target tissues

Background and objectives: PUUV is one of the most important hantaviruses in Europe, with the bank vole being its reservoir. The Robert Koch-Institute registered 1731 human hantavirus infections in Germany during the "hantavirus year" 2017. 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 don't exhibit any signs of disease. Thereby, the target tissues of hantaviruses are poorly characterized in the natural reservoir.
Materials and methods: Carcasses of naturally infected laboratory bank voles and wild bank voles which were trapped during spring and autumn of 2015 till 2017 in the district Osnabrück were dissected looking for gross pathologic findings, and subsequently tested for hantavirus-RNA by reverse transcription-PCR using lung tissue. PUUV-RNA positive animals were tested via hematoxilin eosin staining (HE), indicating inflammatory/degenerative lesions, and immunohistochemistry using a polyclonal swine-anti-hantavirus-nucleocapsid-protein-antibody.
Results: Intracytoplasmatic granular signals, representing the nucleocapsid-protein-expression, were found in brain, heart, lung, stomach, parotis, pancreas, liver, kidney and testes.

Immunopositive tissues had no gross or histopathological (HE) lesions.
Conclusion: To our knowledge, it is the first immunohistochemical characterization of the target tissues of PUUV in bank voles.

Poster Session Public Health

H01

Impact of Roundup, a glyphosate-containing herbicide, on pathogenic Enterobacteriaceae in a 'Rumen Simulation Technique' -fermenter model

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Keywords: glyphosate, Enterobacteriaceae, RUSITEC

Background and objectives: Roundup (RU), a glyphosate-containing herbicide, is currently the most widely used herbicide in the world. Glyphosate targets the enolpyruvylshikimate-3-phosphate synthase present both in plants and in bacteria. This led to the concerns about the effects glyphosate in animal feed may have on intestinal microbiota in farm animals. Here, we investigate whether presence of RU may enrich the rumen microbiota for pathogenic Enterobacteriaceae.
Materials and methods: Six rumen simulation technique (RUSITEC) fermenters populated with normal cow rumen microbiota were inoculated with pathogenic strains of *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. Ten mg/L Roundup LB plus, representative of the „worst-case" exposure regimen in cattle, were added into three fermenters, while the remaining three fermenters served as controls. The experiment continued for seven days. Bacterial counts for *S. enterica and E. coli* were estimated daily by plating on selective agar.
Results: Numbers of colony forming units of both species declined steadily in the control and RU group over time. No *E. coli* could be detected 96 hours after inoculation, while *Salmonella* persisted until day 7 in all fermenters. There was no statistically significant difference in bacterial counts between the control and RU group for both species.
Conclusion: exposure to 10 mg/L RU does not appear to enrich for pathogenic *Salmonella* and *E. coli* in the *in vitro* cow rumen fermentation model.

H02

Comparative Analyses for the Improvement of the DNA Extraction of *Cryptosporidium* for Molecular Detection

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Keywords: Molecular Detection, nested PCR, Cryptosporidium

Background and objectives: Cryptosporidiosis is a widespread diarrheal disease of humans and animals caused by the uptake of the protozoan parasite *Cryptosporidium*. Every year, more than eight million human infections are registered worldwide, although large scale underreporting is suspected. Currently, there is a lack of harmonized molecular methods for sensitive and specific pathogen detection. Therefore, the aim of this study was to compare different methods for the pulping of oocysts to optimize the molecular detection of *Cryptosporidium*.
Materials and methods: Water and faeces were spiked with different numbers of *C. parvum*. Various sample preparation methods (e.g. heat treatment, thawing/freezing, ultrasound, DNA extraction kits, proteinase K treatment) were compared to provide oocysts for nested PCR detection of the specific sequences 18SrDNA, COWP & GP60.
Results: The most efficient molecular detection in water was achieved with prefixed thawing/freezing cycles combined with bead disruption (detection of one oocyst/µl). Preliminary results indicate that the use of DNA extraction kits is the most promising method for the molecular detection of *Cryptosporidium* in faeces (detection of one oocyst/µl).
Conclusion: There are many different methods that were employed to disrupt oocysts of *Cryptosporidium* for molecular detection. The results of this study show that the choice of the method should depend on the matrix. Besides, not all methods are similarly suitable for an effective disruption of the oocysts.

H03

Food safety and food security in Somaliland: a successful implementation of the One Health Approach

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Keywords: One Health, food safety, food security

Background and objectives: Food safety, nutrition and food security are inextricably related and need to be tackled with a holistic One Health approach. In Somaliland, the availability of local animal products is unreliable. The aim of this project is to improve food safety and security by enhancing access of consumers to local high quality and safe animal protein and stabilizing the livelihood of all members of the milk and meat value chain: producers, processors, vendors and Community Animal Health Workers (CAHWs).
Materials and methods: the members of the milk and meat value chain receive technical trainings in hygienic practices and suitable equipment. CAHWs and private veterinary drug suppliers are trained and receive supplies to optimize animal health. Milk centres, slaughterhouses and markets are being constructed. In addition, relevant stakeholders in the public sector are provided with capacity building and support, to contribute to policy development and implementation.
Results: The hygiene and quality of local animal products has improved substantially, hence increasing the income of producers, vendors and processors due to increased milk prices (from 0,8 to 1,25 USD). Moreover, the access of the population to hygienically improved animal protein is better.
Conclusion: The One Health approach has proved to be an essential strategy to accomplish the objectives of this project, whose impacts come from an integrative transdisciplinary intervention at all levels.

H04

Tenacity of Clinical Relevant Surrogate Pathogens in Aerosolized Conditions

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Keywords: Aerosol, Tenacity, Humidity

Background and objectives: To investigate the decontamination of medical equipment and surfaces after pathogen exposure, clinically relevant surrogate pathogens were examined in an aerosol chamber at different humidity levels. This was done to ascertain if the humidity has any influence on the recovery rate and the tenacity of the aerosolized strains.
Material and methods: Biological strains used: *Staphylococcus (S.) aureus* as a surrogate strain for airborne bacteria and *Geobacillus (G.) stearothermophilus* as a strain for spore forming bacteria. The experiments took place in an aerosol chamber with adjustable climate parameters. The amount of Colony forming Units (CfU) were set for 1E+06 CfU per cubic meter of air. The tested humidity was between 30% and 70% Relative Humidity (RH).
Results: While the results from *G. stearothermophilus* spores show a tenacity that is independent from the tested humidity, *S. aureus* shows an increase of tenacity for humidities that are lower than 50% RH.
Conclusion: The stability of *G. stearothermophilus* spores in comparison to the *S. aureus* can be explained by the general stability of spores to environmental influences. The results for *S. aureus* were not reported before. Future studies should investigate why the bacteria show an increase of tenacity in air at low humidity.

H05

Staphylococcus aureus in the dairy food chain in Zambia (SAD-Zambia project)

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Keywords: MRSA, milk, Africa

Food security in African countries can only be improved if food is safe. Milk in particular has been considered the "ideal food", especially for children, but it can be also a source of zoonotic bacteria, such as *Staphylococcus aureus*.
The SAD-Zambia project aims on characterising and reducing the health risks experienced by consumers and producers related to *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), in the dairy food chain in Zambia. For that, 320 facilities (i.e. farms, milk collection centres, traders, processing plants, traditional markets, and supermarkets) were visited in three regions of Zambia to collect samples of different matrices (milk and milk products; nasal, hand, bucket swabs; water). From the 2,162 samples, 379 presumptive *S. aureus* strains were isolated so far. Of these, 351 have been analysed by MALDI-TOF and 295 are confirmed as *S. aureus*.
The *S. aureus* isolates will be tested for the presence of a *mecA* (or *mecC*) gene to identify MRSA and selected strains will be further characterised regarding virulence and antibiotic resistance.
Preliminary results indicate a wide distribution of *S. aureus* in the traditional Zambian milk chain, including unprocessed, pre-processed, and sour milk. In contrast, no *S. aureus* could be isolated from processed milk samples (heat-treated milk, sour milk, and cheese) at retail level, indicating the efficacy of industrial processing to reduce *S. aureus* in milk (products) in Zambia.

H06

Detection of *Rickettsia amblyommatis* in an *Amblyomma mixtum* nymph that infested a German female traveller returning from Cuba

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Keywords: Amblyomma mixtum, Rickettsia amblyommatis

A partially engorged *Amblyomma mixtum* nymph was found on a healthy female traveller from Germany upon her return from a visit to Cuba. DNA was extracted from the nymph and a blood sample and subjected to PCRs amplifying the rickettsial 16S rRNA and *ompA* genes. Whereas the blood sample tested negative by PCR, sequence analysis of the 16S rRNA and ompA amplicons from the tick revealed 100% identity with *Rickettsia amblyommatis*, a recently described spotted fever group Rickettsia which is highly prevalent in *Amblyomma* ticks in the Americas. Although there is evidence that *R. amblyommatis*, sometimes also reported as *R. amblyommii*, is associated with disease manifestations in some patients, the woman did not develop an eschar or clinical disease.

H07

Environmental occurrence of *Campylobacter* spp. in broiler farms and surroundings

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Keywords: campylobacter, tenacity, environment

Background and objectives: Emission of *Campylobacter* spp. into the environment has been known as major public health and economical concern. Nevertheless, the occurrence and tenacity of *Campylobacter* spp. in broiler farm surroundings is remained unclear. As part of the PAC-Campy consortium, we study the prevalence of *Campylobacter* spp. in broiler farms and their survival in surrounding areas.
Materials and methods: *Campylobacter*-positive broiler farms are selected by an initial screening (boot swabs and pooled faeces samples). These farms are sampled intensively by taking boot swabs, air samples and swab samples from various surfaces both inside the barn and in the environment. To measure the prevalence inside the barns pooled faeces are gathered. Sampling will take place during summer and winter time. At each farm two consecutive fattening periods and the efficacy of cleaning and disinfection are investigated. All Samples are processed based on DIN ISO 10272 for *Campylobacter* spp. and positive samples were further analysed with Mass spectrometry and multiplex PCR assay.
Results: The first screenings proved two investigated farms to be positive for *Campylobacter* spp. At farm A, all 4 barns and at farm B one out of four barns were tested positive. Mass spectroscopy and PCR identified *C. jejuni* in all positive samples.
Conclusion: The investigations will help to better understand the significance of *Campylobacter* spp. in the broiler farms surroundings for spread and re-entrance.

H08

Establishing a seeder bird model to evaluate the effectiveness of non-biosecurity based measures on *Campylobacter* prevalence in broiler flocks

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Keywords: campylobacter, seeder bird, non-biosecurity based measures

Background and objectives: Previous approaches to reduce Campylobacter infections in broiler farms have focused mostly on improving biosecurity measures. However, these measures have been proven to be insufficient. Thus, in this study, we aim to examine the effectiveness of non-biosecurity based measures on Campylobacter prevalence in animal trials.
Materials and methods: To establish a *Campylobacter* colonisation model broilers of breed ROSS 308 will be fed with standard diet and retained on ground floor with litter and a stocking density of 39 kg/m². We will start determining the dose necessary for oral infection using $1x10^1$ to $1x10^5$ colony forming units of a *C. jejuni* reference strain on day ten of live. The dose that results in a colonisation rate higher than 95 % at necropsy will be used for the following investigations. In the second part, we will test the seeder bird model in a 1:5 ratio to define the number of animals, which have to be colonised initially. Successful colonisation will be validated by cloacal swabs once a week and by investigation of the intestinal content at the end of the trial during necropsy. Samples will be analysed for *Campylobacter* spp. by quantitative and qualitative bacteriological investigation according to DIN ISO 10272.
Conclusion: When the colonisation model is established we want to investigate the effectiveness of different non-biosecurity based measures on the *Campylobacter* spp. prevalence of broilers at the end of fattening.

H09

In-vitro Study on the antimicrobial effect of organic acids on *Campylobacter* spp.

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Keywords: Campylobacter spp., organic acids, broth-microdilution

Campylobacteriosis is one of the most important foodborne gastrointestinal diseases in Europe. It is assumed that contaminated poultry meat is the major single source of infection with *Campylobacter* spp.. A reduction of the bacterium in primary production of broiler meat is considered to be most effective to combat this disease. Different *in-vitro* and *in-vivo* studies revealed the antibacterial potency of some organic acids.
In this study, the antimicrobial activity of formic, acetic, propionic, ascorbic and tartaric acid were tested against 30 field isolates of *Campylobacter* (*C.*) *jejuni* and *C. coli.* The minimum inhibitory concentrations (MIC) were determined using a broth-microdilution assay. Inoculum level, growth media, and incubation times followed Clinical and Laboratory Standards Institute (CLSI) guidelines. The test ranges for all organic acids included the following concentrations: 1-1024 mmol/l. The tests were performed at pH 6.0 and 7.3 to assess the impact of the pH value on antimicrobial efficacy.
Most organic acids had a stronger antibacterial effect at pH 6.0. The *Campylobacter* spp. isolates showed different levels of susceptibility to the tested substances. The lowest MIC values were detected for ascorbic acid, while tartaric acid had a weak antimicrobial activity.
The results of this study might contribute to the development of a water additive to reduce *Campylobacter* spp. in poultry.

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Poster Session Free Topics

F01

Identification of immunodominant proteins of *Brucella canis* using sera of naturally infected dogs

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Keywords: Canine brucellosis, serological diagnosis, immunoproteomics.

Background and objectives: Canine brucellosis is a zoonotic disease that may cause reproductive failure in dogs. The infection is frequently misdiagnosed using classical microbiology and serological tests. To improve laboratory diagnosis we identified proteins of *Brucella canis* specifically binding antibodies of naturally infected dogs.
Materials and methods: Within an outbreak of canine brucellosis in a kennel from São Paulo (Brazil), a total of 17 adult pugs and 5 beagles were grouped into infected (bacteremic or non-bacteremic) or non-infected dogs according to serial microbiological and serological test results. Whole-cell protein extracts of *B. canis* isolates from infected dogs were separated using 2D gel electrophoresis. Western blotting was performed with all sera (n = 56) collected from infected and non-infected animals. Immunogenic protein spots were characterized by mass spectrometry.
Results: We were able to identify 93 different immunogenic proteins, with 19 only found in infected dogs (bacteremic as well as non-bacteremic) but not in *Brucella*-free dogs. Most of the proteins were ion transporters or involved in metabolism.
Conclusion: Infected and non-infected dogs can be differentiated by specific immunogenic proteins. Immunological patterns may also help to diagnose the stage of infection. For this purpose, the 19 differentiating proteins identified must be further evaluated as antigens in serological tests.

F02

Towards establishment of a model to study in vitro reassortment and adaptation of human H1N1pdm09 and swine influenza A viruses

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Keywords: influenza A virus, reassortment, H1N1-2009 pandemic.

Background and objectives: Since 2009, the "Swine flu" pandemic H1N1pdm viruses have fully replaced the previously circulating H1N1 lineage in the human population. Moreover, they have been re-introduced into the swine population, generating a variety of reassortants in combination with circulating swine influenza viruses (SIVs). This phenomenon is of public health concern, since it is unknown whether these variants may pose a risk to humans. Our goal is to study reassortment processes between human H1N1pdm09 and SIVs in different hosts *in vitro*.
Materials and methods: To establish a model to study reassortment and adaptation, replication abilities of H1N1pdm09 and SIVs (H1N1, H3N2, H1N2) were analysed in three different swine lung cell lines and in human lung A549 cells. After co-infection and passaging in swine and human cells, genome composition of viral progeny will be analysed by deep sequencing.
Results: H1N1pdm09 replicated in WSL-R-HP and WSL-R-584 swine cell lines, whereas SLU-R cells appeared to be non-permissive for this strain. Furthermore, H1N1pdm09 and the SIVs were able to replicate in both selected host models: human A549 and swine WSL-R-HP cells, even at low multiplicity of infection.
Conclusion: We identified a suitable swine cell line as well as a human cell line for H1N1pdm09 reassortment studies. Moreover, all IAVs tested did not require adaptation prior to infection of a new host, highlighting the zoonotic potential of these strains and their reassortants.

F03

Inhibition of Borna disease viral spread by a furin inhibitor *in vitro* and *in vivo*

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Keywords: Borna disease virus 1, furin inhibitor

Background and objectives: Due to the recently reported cases of Borna disease virus 1 (BoDV-1) transmission through organ transplantation antiviral strategies are definitely needed. It has been shown that the viral surface glycoprotein, after its activation by the ubiquitary host protease furin, has an important role in the life cycle of BoDV-1 and determines viral spread and enables virus persistence by tightly regulated expression.
Materials and methods: A highly potent furin inhibitor (MI-0701) was used in several primary but also permanent cell culture systems to test its inhibiting potential on viral spread. In an *in vivo* experiment immunocompetent Lewis rats were intranasally infected with BoDV-1 and simultaneously treated with the furin inhibitor, mimicking the natural route of infection. Immunohistochemistry and immunofluorescence has been used to detect viral antigen.
Results: Mixed neuronal derived cultures, cultures of olfactory epithelium and those of pure olfactory ensheathing cells showed a dose-dependent decrease of infection up to 60% compared to non-treated cells. *In vivo* the olfactory epithelium as well as the brain tissue demonstrated a distinct decrease of BoDV-1-antigen with accentuation on bulbus olfactorius, hippocampus and the entorhinal cortex after 21 days p.i.
Conclusion: This furin inhibitor can reduce viral spread *in vitro* as well as *in vivo* in acute infection stages, its impact on persistent infection has to be further analysed.

F04

Investigating the Zoonotic Origin of Human Metapneumovirus

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Keywords: avian Metapneumovirus, human Metapneumovirus, phylogeny

Background and objectives: Avian Metapneumovirus (aMPV) causes substantial economic losses for the poultry industry and is one of only two members of the genus *Metapneumovirus*, the second being human Metapneumovirus (hMPV), a leading cause of acute respiratory disease in children. The genome sequence of aMPV subtype C, in particular, is similar to that of hMPV, suggesting that this human pathogen has a zoonotic origin via an historical cross-species event. Our aim is to obtain a better understanding of the underlying molecular determinants which facilitated the establishment of an avian virus in the human population.
Materials and methods: In this study we screened approx. 500 swab samples from wild birds in the Netherlands, using RT-PCR with primers targeting all Paramyxo- and Pneumoviruses. We obtained the full length genome sequence of a newly identified aMPV-C via next generation sequencing. Maximum likelihood phylogenetic analysis using MEGA 7 enabled comparison of this strain to other metapneumoviruses.
Results: The newly obtained sequence shows a 97% nucleotide identity to both Chinese strains S01 and GDY. Our phylogenetic tree shows a closer relationship to hMPV than to aMPV subgroups A, B, D, with all strains of subgroup C clading with hMPV strains.
Conclusion: The first full genome sequence of a wild-type aMPV-C strain will facilitate the generation of a reverse genetics system to enable mechanistic investigations into the evolutionary origin of hMPV.

F05

Eight years of astrovirus monitoring in German bats: New insights into bat astrovirus characteristics

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Keywords: bat-astrovirus, phylodynamics, monitoring

Background: Astroviruses (AstVs) are small single stranded RNA viruses present in several avian and mammalian species. AstVs may cause asymptomatic infections, but are also correlated with symptoms ranging from diarrhoea, nephritis, hepatitis, respiratory syndrome to encephalitis, dependant on variant and host species. Little is known about the characteristics of AstV infection in bats. As bats are endangered species in Germany, most classical assays on those interactions are not feasible.
Materials and Methods: We collected fresh faecal and urine samples during a monitoring program on individually tagged bats at up to three distinct locations in Germany. These samples, partly collected since 2011, were screened using a hemi-nested RT-PCR for the presence of AstV RNA and the variants were identified by Sanger sequencing.
Results: Four analysed bat species showed distinct characteristics regarding the amount of different AstV variants being detected and their detection rates. The composition of detected virus sequences varied strongly between the years. Several new variants were detected. Individuals being screened at different consecutive timepoints give first hints on a potential virus clearance.
Conclusions: This study monitors the AstV presence in different populations of tagged and thus identifiable German bats over time and thereby reveals distinct bat AstV variant transmission dynamics where regular experiments would not be possible.

F06

Highly pathogenic H5N1- and H5N8-type avian influenza A virus (IAV) strains: Impact of possible reassortment with co-circulating Eurasian H9N2-type avian IAV on viral replication and pathogenicity in mammals

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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/H5N1, 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 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/H5N1$_{(EGY)}$ or HPAIV/H5N8$_{(GER)}$. Based on our results we discuss the influence of specific reassortments on the zoonotic potential of Egyptian HPAIV/H5N1 and German HPAIV/H5N8 in mammals *in vitro* and *in vivo*.

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F07

Effect of duration of formalin fixation on RNA quality

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Keywords: Formalin fixation, RNA isolation

Background and objectives: Around the world, pathology archives represent a valuable source for tissue material from clinical cases with known/defined clinical history. Usually, these tissues are stored in the form of formalin-fixed paraffin embedded tissue (FFPE) with a routine formalin fixation time of 1-2 days before paraffin embedding. Under high biosafety conditions, this fixation time is extended up to three weeks. It is known that the fixation induces degradation and fragmentation of RNA, however, the effect of the duration of fixation time on the RNA quality is not known.
Materials and methods: RNA was isolated from tissue samples of brain, kidney, liver and muscle, that were fresh, underwent formalin fixation for 24h, 48h, 7 days, 14 days and 21 days or were stored at -80°C for 3 weeks. Amount of isolated RNA and fragments lengths were compared.
Results: Beside the differences in RNA quality gained from fresh or frozen samples compared to FFPE material, there was no difference between the quality of short fixed and long fixed material.
Conclusion: Prolonged formalin fixation under high biosafety conditions doesn't impair the quality of RNA gained from FFPE material compared to formalin fixation under standard conditions.

F08

Characterization of clinical equine *Staphylococcus aureus* isolates with reduced susceptibility to oxacillin

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Keywords: antimicrobial resistance, whole genome sequencing, MLST

Background and objectives: In human and veterinary medicine methicillin resistance among staphylococci is a major threat. Therefore, equine *Staphylococcus aureus* isolates with reduced susceptibility to oxacillin were characterized.
Materials and methods: From 2015 to 2017, 19 isolates of an equine clinic with reduced minimum inhibitory concentrations (MICs) for oxacillin via VITEK2 (0.5 to ≥ 4 mg/L) were investigated. The isolates were subjected to whole genome sequencing (WGS) and the Multilocus sequence (MLST) and *spa* types were deduced. Antimicrobial susceptibility testing was performed by broth microdilution according to CLSI. The respective resistance genes were detected in the WGS.
Results: Two MLST and four *spa* types were detected: ST1 (n=3) all with *spa* type t127, and ST1660 isolates with the related *spa* types t3043 (n=14), t549 (n=1) and t2484 (n=1). All isolates lacked the methicillin resistance genes *mecA* and *mecC*. All isolates were resistant to penicillins [*blaZ*], gentamicin and kanamycin [*aacA-aphD*, *aadD*]. The ST1 isolates were additionally resistant to tetracyclines [*tet*(L)] and the combination trimethoprim [*dfrG*] and sulfamethoxazole. Broth microdilution revealed lower oxacillin MICs of 0.25-0.5mg/L of the ST1 isolates compared to the ST1660 isolates with oxacillin MICs of 1-2mg/L.
Conclusion: The *S. aureus* isolates belonged to two different sequence types and had different resistance properties, with the ST1 isolates displaying a multiresistance phenotype.

Poster Session Bioinformatics

B01

Bioinformatics analysis of whole genome sequences of *Francisella tularensis* isolates generating new insights into tularemia in Germany

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Keywords: Francisella tularensis, whole genome sequencing, bioinformatics

Background and objectives: *Francisella* (F.)*tularensis* is a highly virulent, Gram-negative bacterial pathogen and the causative agent of the zoonotic disease tularemia. These allowed evaluating and establishing a diagnostic procedure for Germany. Evaluation and

Materials and methods: Field isolates that caused fatal tularemia in German hare (*Lepus europaeus*) were isolated characterized and sequenced. High quality circular genome sequences of the *F. tularensis subsp. holarctica* were generated, analyzed and characterized. Besides the genomic structure, the analysis of an oriC, unique to the *Francisella* genus and the genomic DNA and a unique methylation pattern was analyzed. Additionally whole genome sequences from *F. tularensis* isolated from Germany were generated. Results: Bioinformatics methods were evaluated and established for next generation sequences. The phylogenetic analysis with different tools for genotyping allowed establishing a genotyping strategy for *F. tularensis*. These methods imply to assign microbial phylogeny and putative taxonomy using proteins (PhyloPhlAn) and SNPs (Parsnp) proved to be versatile in the epidemiological assessment of *F. tularensis* subsp. *holarctica*.

Conclusion: A diagnostic whole genome sequencing pipeline was established and evaluated. This novel analysis allows a detailed classification, a very precise placement and the utilization of readily available whole genome data, independent of databases and reference genomes.

B02

Population structure and neurotoxin subtype distribution in *Clostridium botulinum* group I and *Clostridium sporogenes*

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Background and objectives: Historically, the name "botulinum" was assigned to *Clostridium* strains producing the neurotoxin, independently from the phylogenetic background. The major aim of our study was to analyse a comprehensive collection of *C. botulinum* group I and *C. sporogenes* in order to identify the correct taxonomic assignment of each isolate and to provide a more robust picture of the overall population structure of these two closely related species as well as the neurotoxin subtype distribution.
Materials and methods: 84 newly sequenced genomes and 136 publically available whole-genome sequences of *C. botulinum* and *C. sporogenes*, were used. Isolates with an average nucleotide identity higher than 95% were regarded as the same species. Phylogenetic analysis was performed using Roary and RAxML software, while neurotoxin subtype distribution was assessed by means of an *in silico* Megablast-based approach.
Results: Three major clusters and three different species, namely *C. botulinum* group I, *C. sporogenes sensu stricto* and *C. sporogenes sensu lato*, were observed. Misclassified isolates were identified. Both species include members producing or not the neurotoxin. For *C. botulinum* a correlation between neurotoxin subtype and phylogentetic background was observed. Conclusion: This work contributes to a better understanding of *C. botulinum group I and C. sporogenes* population structure in terms of genetic diversity and neurotoxin distribution.

Poster Session Parasites

S01

Characterization of the contribution of phenotypic heterogeneity to the persistence of *T. gondii*

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Keywords: phenotypic heterogeneity, Toxoplasma gondii, Life cell Imaging

T. gondii is an apicomplexan parasite that infects all warm blooded animals and causes chronic infections in 50% of the German population. Current medical treatments target fast replicating tachyzoite stages but exert severe side-effects and fail to clear chronic infections. In contrast to tachyzoites, these persisting bradyzoites are contained in morphologically diverse tissue cysts and are marked by asynchronous cell divisions.
To investigate whether this phenotypic heterogeneity presents a bet-hedging strategy and is associated with treatment failures we aim to characterize the physiological diversity of these cysts. To this end we established a novel protocol that allows us to culture drug-resistant cysts over weeks in their natural host cell types. We construct a panel of fluorescent reporter cell lines expressing genetically encoded FRET-based fluorescent metabolite sensors and fluorescent cell cycle reporter proteins. Using life-cell imaging we explore the diversity of physiological states and their susceptibility towards external stressors such as antimicrobials with known and unknown modes of action in different veterinary isolates.
This project improved in vitro access to chronic *T. gondii* stages significantly and enables detailed studies to understand how phenotypic diversity can translate into persisting pathogen populations as observed in many zoonotic infections.

S02

Frequency and Distribution of *Ixodes frontalis* in Germany

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Keywords: Ixodes frontalis, avian tick-related-syndrome, ornitophile ticks

The ornithophile tick species *Ixodes frontalis* (Panzer 1798) is present in Europe, Northern Africa and Asia. It can be a carrier of different bacteria and viruses and transmit some species of the *Borrelia burgdorferi* s.l. group. In addition, it is the causative agent of the "avian tick-related-syndrome" that can commonly result in death of infested passerine and psittacine birds.
As it is common for ornitophile ticks, *I. fronatlis* is usually collected directly from host animals and their nests. Detections resulting from the usage of the flagging method are hardly known. In Germany, the species was considered lost for a long time, causing Schulze to state in 1933 that "the species has not been re-discovered again since the time of Panzer". It has however been detected in Wiesbaden on a rose-ringed parakeet in 2007 and by flagging in Ingolstadt in 2011.
In this study, we report the detection of 1046 individuals of the species *I. frontalis* collected with the flagging method originating from the urban areas of Stuttgart, Mannheim and Hannover. In addition, five larva of the species could be collected with a Berlese funnel from foliage of a garden in Hohenheim. Furthermore 177 Passeriformes from Germany were screened for ticks. In total, 123 *I. frontalis* were detected on 35 infested black birds.

S03

In vitro investigation of oocyst-specific proteins for their role in survival of *Toxoplasma gondii* oocysts in the environment

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Keywords: stress tolerance, oocysts, desiccation (max. 3)

Background and Objective: Four oocyst-specific proteins have been annotated as so called 'late embryogenesis abundant domain-containing protein' in the genome of *Toxoplasma gondii* (TgLEAs). These proteins have characteristics of 'intrinsically disordered proteins'. Since such proteins adopt defined tertiary structure only under specific stress conditions our objective is to investigate whether TgLEAs contribute to protection from desiccation and low temperatures.
Materials and Methods: Each of the TgLEA proteins was cloned and expressed in *Escherichia coli*. Bioinformatic and biochemical analyses were performed to substantiate TgLEAs' classification as intrinsically disordered proteins. In growth assays the impact of each TgLEA on *E. coli* survival after desiccation or incubation at low temperatures was analysed.
The same assays were conducted in five different *Saccharomyces cerevisiae* strains that have different stress-response related gene knock-outs.
Results: Bioinformatic and biochemical analyses strengthened our assumption that TgLEAs are highly intrinsically disordered proteins. Out of four investigated TgLEAs, at least one showed a beneficial effect on *E. coli* viability after desiccation, implying an involvement in desiccation tolerance of oocysts.
Conclusion: TgLEAs that aid in protection against desiccation and low temperatures might contribute to the long survival of *T. gondii* oocysts in the environment and are thus potential targets for novel disinfectants.

S04

Genetically encoded functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor

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Keywords: Arginine deiminase, Giardia duodenalis, virulence factor

Background and objectives: Depleting arginine is a recognized strategy of pathogens to evade immune effector mechanisms and arginine depleting enzymes are considered virulence factors. An arginine deiminase (ADI) has been implicated in the virulence of *Giardia duodenalis*, an intestinal parasite that infects humans and animals causing significant morbidity. Here the hypothesis was tested that sequence variation detected between *G. duodenalis* ADI alleles affects functional parameters of the enzyme.
Materials and methods: Different alleles of *G. duodenalis* ADI were cloned and purified in recombinant form and the enzymes' Km values were determined in vitro. In addition, Km values of *G. duodenalis* ADI activities were determined in lysates of recent clinical parasite isolates and the respective *adi*-genes were sequenced.
Results: We show that sequence variation correlated with changes in Km values of the respective enzyme variants.
Conclusion: Our data add a further molecular argument to the concept of *G. duodenalis* ADI being a molecularly defined virulence factor of *G. duodenalis*.

S05

Long-term signature of insectary adaptation in *Aedes albopictus*

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Keywords: Lab population, field population, Aedes albopictus

Background: Mass rearing of *Aedes albopictus* under laboratory conditions is crucial e.g. for sterile insect technique and genetic vector control approaches. The objective of the present study was to identify how a long-term adaptation to insectary conditions manifests in the phenotypic signature of *Ae. albopictus*.
Materials and methods: A lab strain (LAB, Biogents®) and a wild type strain (WILD, Ravenna, Italy) of *Ae. albopictus* were compared at the genetic (microsatellites, COI), epigenetic (global DNA methylation), physiological (size specific caloric contents of protein, lipids and glycogen) and morphological (nine landmark-based wing parameters, five head parameters) level.
Results: We identified physiological and morphological plasticity between lab and wild type *Ae. albopictus* strains, but no (epi-)genetic differentiation was measured. LAB larvae and pupae metabolized larval food in a better way; however, adults do not profit thereof. In addition, in LAB adults wing morphology is at slightly higher variance, the maxillary palpi are shorter and the body size of LAB females is slightly increased.
Conclusion: In fact, the LAB strain showed a better adaptation to insectary conditions, but only in early life stages. Furthermore, the

slight morphological differentiation in adults may imply a degraded sensory capacity of the LAB strain, which might become a disadvantage when released into a field setting.

S06

Baylisascaris procyonis in free-ranging raccoons *(Procyon lotor)* in Saxony, eastern Germany

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Keywords: raccoon, Baylisascaris procyonis, Procyon lotor

The raccoon is an introduced species in Germany. In their native region, raccoons carry the zoonotic nematode *Baylisascaris procyonis*. *B. procyonis* can produce parasitosis in the brain of paratenic hosts: mammals and birds. Humans can be infected by oral infection of embryonated eggs. In Germany, *B. procyonis* prevalence varies. In Hessen, there is a reported 71% of *B. procyonis* prevalence in raccoons. And *B. procyonis* is absent from northeastern Germany. The objective of this ongoing study is to investigate the prevalence of *B. procyonis* in free-ranging raccoons from the eastern state of Saxony. This abstract presents some of the preliminary results. Methods and preliminary results: From autumn 2017 to spring 2018, hunted raccoon carcases were collected from Leipzig metropolitan area, northwestern Saxony, as well as surrounding regions. The entire gastro-intestinal track, including any faecal content, is removed. Intestines are complete dissected, large nematodes are collected, and morphological identification of *B. procyonis* is conducted. Molecular identification was performed by PCR. So far, 27 raccoon carcasses have been examined and *B. procyonis* has been found in 22 animals.

Conclusions and prospective outcome: We report the presence of *B. procyonis* in raccoons from the Leipzig area. Since raccoons are commonly attracted to anthropogenic settlements, the risk of human infection is considerable; particularly for wildlife professionals, veterinarians, and hunters.

S07

Eradication of Human African Trypanosomosis? Impossible without a One Health approach!

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Animal African Trypanosomosis (AAT) and Human African Trypanosomosis (HAT), caused by a protozoa of the genus *Trypanosoma* (section Salivaria), are both diseases of significant importance to sub-Sahara Africa. Several species of *Trypanosoma* are found in Africa but only two subspecies of *T. brucei*, are relevant to humans. They either cause the chronic (*T. b. gambiense*) or the acute (*T. b. rhodesiense*) form of human sleeping sickness, both with a fatal outcome if left untreated. While wildlife have long been known to be reservoirs for both HAT and AAT and show no clinical symptoms, infection in livestock, especially cattle, causes severe losses to local producers and are potential reservoirs for rhodesiense-HAT. Domestic pigs, too, are preferred hosts of *Glossina* spp. and have been reported to be potential reservoirs of both forms of HAT. Pigs are increasingly important as a source of income and food for smallholder livestock farmers in East Africa, especially in Uganda where both forms of HAT as well as AAT are endemic. Except for *T. suis* and *T. brucei gambiense*, all *Trypanosoma* species known to infect pigs have been reported from pigs in Uganda. The authors present findings from a review on the potential role of pigs as a livestock reservoir for HAT. We will discuss how and why infection with *Trypanosoma* spp. in pigs should be considered in differential diagnoses in clinically sick animals as well as in national HAT surveillance and eradication programs.

S08

CESSARi: an initiative for research on cystic echinococcosis in sub-Saharan Africa

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Background and objectives: Cystic echinococcosis is widespread in sub-Saharan Africa. Despite recent considerable research efforts, available data largely concentrated on few foci (e.g. Turkana region in Kenya), while some existing data were difficult to access. The aim of CESSARi was to identify local scientific expertise, create and strengthen research facilities, mobility and communication between African institutions. An extensisve network was established and addressed open questions concerning presence, frequency and genetic identity of CE agents in different African countries.
Materials and methods: From 2009 to 2018, the DFG-funded network included thirteen medical, veterinary and biological research institutions in Sudan, Ethiopia, Kenya, Uganda, Zambia, Namibia, South Africa and Germany.
Results: CE prevalence in humans and livestock was re-assessed or newly established. All five species of *Echinococcus* that cause CE were found in sub-Saharan Africa. The distribution of the various taxa was not homogenous, high prevalence of human CE correlated with the presence of *E. granulosus* sensu stricto. Lifecycles of most *Echinococcus* species were found to involve wild animals to a previously unknown extent.
Conclusion: The established network was efficient in gaining new insights in *Echinococcus* epidemiology in a neglected region as well as strengthening research capacities in Africa and cooperation pathways within the continent.

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