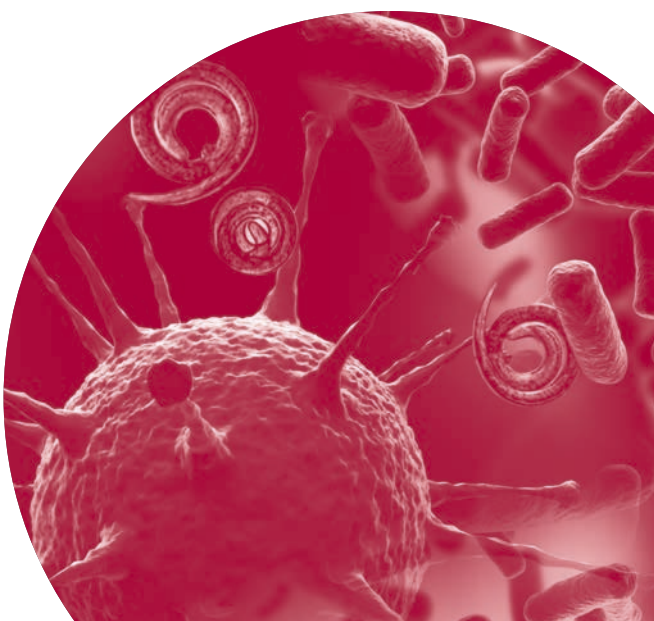
Nationale
Forschungsplattform
für Zoonosen

National Symposium on Zoonoses Research

13 – 14 October | Berlin 2016

Program and Abstracts



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Nationale
Forschungsplattform
für Zoonosen

Editor

German Research Platform for Zoonoses

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Institute of Novel and Emerging Infectious Diseases (INNT)
Südufer 10, 17493 Greifswald–Insel Riems, Germany

Table of Contents

Welcome Address of the German Research Platform for Zoonoses

Dear colleagues,

We are delighted to welcome you to the National Symposium on Zoonoses Research 2016.

Zoonoses are a global challenge – whether they are well-known or newly emerging diseases, whether they are caused by viruses, bacteria, parasites or prions, or whether they are transmitted directly or via vectors. The current Zika virus outbreak in the Americas underlines the huge impact zoonoses can have on humanity, with consequences that go far beyond the obvious health concerns. Well-founded interdisciplinary research is essential to successfully fight this health burden.

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 Thursday morning and Friday afternoon (including a talk on the aforementioned Zika virus research) and as well as a special Data Management session on Thursday.

We would like to take this opportunity to thank everyone who has submitted abstracts and 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 idea to life!

Stephan Ludwig
(Münster, Germany)

Martin H. Groschup
(Isle of Riems, Germany)

Sebastian C. Semler
(Berlin, Germany)

Directors of the German Research Platform for Zoonoses

Welcome Note of the Federal Ministries

Fostering scientific research for a better understanding of zoonotic diseases and their control has been a priority of the Federal government of Germany for more than a decade now. The important results from the numerous projects and their implications for the protection of human and animal health demonstrate the success of zoonoses research in Germany over the past decade. Consequently, the three competent Federal Ministries of (Research and Education, Health, Agriculture and Nutrition) have renewed their common Zoonoses Research Agenda early this year, taking on board the Federal Ministry of Defence as a new partner.

The 'One-Health' approach, the central principle of the joint Research Agreement, expresses our firm conviction that both human and animal health are closely linked with one another. Zoonoses research that aims at supporting Public Health and veterinary health authorities needs a common approach and a close collaboration of the different domains. Only a comprehensive and interdisciplinary approach will allow for the efficient control of zoonotic diseases and the development and implementation of effective political measures.

Recent challenges that demonstrate the urgent need for this interdisciplinary cooperation are numerous. The ongoing Zika virus epidemic in the Americas, for example, has revealed the hitherto unknown risks posed by a well-known *Flavivirus*. The rapid spread of the disease, which has now reached North America and is at the doorstep to entering Europe, impressively demonstrates the need for continued efforts in research on vector-borne diseases and vector control. This recent outbreak underlines the necessity of seemingly old-fashioned virtues such as vector control in our globalised world and the German federal Government is grateful for the professional guidance by the German research community, allowing for the practical implementation of effective measures.

On the other hand, our needs to foster cutting-edge science and technology for Public Health purposes can be demonstrated in the context of our efforts to improve food safety. Only recently, a *Listeria* outbreak in Germany was resolved using modern genome sequencing technology. Tracing back a zoonotic pathogen from patients to products and their origin at the genetic level provides new opportunities to improve food safety and creates new synergies for the cooperation of Public Health, veterinary and food safety authorities. Examples like these provide a clear idea about what is needed for Public Health and Public Veterinary authorities in highly

developed countries like Germany. Facilitating cooperation and exchange of knowledge beyond scientific disciplines was the original objective for the creation of the The National Research Platform for Zoonoses. One aspect of the successful work of this platform, highly appreciated by the federal ministries, is its function as an efficient exchange forum for scientists and Public Health representatives. Another important aspect of the collaborative work within the Research Platform is the furtherance of young scientists. As in previous years, young researchers will be given the possibility within the context of this symposium to share the results of their work and to discuss these with the attending national and international senior researchers.

Despite all progress, the fight against infectious diseases remains a vast and global challenge for human and veterinary medicine. Quickly developing situations like epidemics caused by highly pathogenic zoonotic agents require fast and reliable response from researchers, health care professionals and regulators. With these challenges and the specific needs of the different actors in mind, the German Federal Government will continue to provide the support that is required to maintain the best possible conditions and structures for excellent research and its translation into practice.

Therefore, we wish all participants at the National Symposium on Zoonoses Research 2016 interesting discussions, new insights, creative ideas for future research initiatives and a successful conference!

Jürgen Thelen, MSc, MPH
(Federal Ministry of Health)

Dr. Joachim Klein
(Federal Ministry of Education and Research)

Dr. Ralf Rotheneder
(Federal Ministry of Food and Agriculture)

Dr. Nicola Wolff
(Federal Ministry of Defense)

Program

Thursday, October 13, 2016

08:00	**Registration (Poster Mounting)**
10:00 – 12:00	**Plenary Session** **(Room Ballsaal)** Chair: *Stephan Ludwig*
10:00	Opening Remarks
10:30	**Keynote 1:** **Complexity, Networks and Disease Dynamics** Dirk Brockmann, Berlin, Germany
11:15	**Keynote 2:** **AIDS, Avian flu, SARS, MERS, Ebola, Zika** **...what next?** Ab Osterhaus, Hannover, Germany
12:00	*Lunch and Poster Viewing*
14:00 – 15:30	**Session 1: Antimicrobial use and resistance I** **(Room Ballsaal)** Chairs: *Birgit Walther and Hendrik Scheinemann*

14:00 **Whole-genome sequence analysis of ESBL-producing *E. coli* of ST410 from avian wildlife, human, companion animal and environmental origins indicates interspecies transmission**
K. Schaufler, M. Wöhrmann, R. Baddam, K. Müller, P.Gastmeier, B. Kohn, T. Semmler, L.H. Wieler, C. Ewers, **S. Guenther**

14:15 **Cattle as a source of *Acinetobacter baumannii* and Carbapenemase-positive *Acinetobacter indicus***
P. Klotz, S. Göttig, U. Leidner, T. Semmler, S. Scheufen, C. Ewers

14:30 **Antibiotic usage in livestock in Germany - Which drug classes are used?**
S. Kasabova, M. Hemme, L. van Rennings, M. Hartmann, I. Ruddat, C. von Münchhausen, A. Kaesbohre2, L. Kreienbrock

14:45 **Disrupting the fatal interplay of influenza virus and pneumococcal neuraminidases by dual-acting inhibitors**
A. Hoffmann, M. Richter, E. Walther, Z. Xu, L. Schumann, U. Grienke, C.E. Mair, J. Kirchmair, S. von Grafenstein, K.R. Liedl, V. Makarov, S. Nietzsche, W. Pfister, J.M. Rollinger, **M. Schmidtke**

15:00 **Harmonisation of biocide susceptibility testing of bacteria**
A. T. Feßler, F. Geber, M. Meurer, V. Hensel, S. Speck, M. Reinhardt, G. B. Michael, U. Truyen, S. Schwarz

15:15 **Characterization of the phenotypic antimicrobial resistance and biofilm profile of mastitis-associated bacteria**
J. Assmann, T. Janßen, C. Fidelak, C. Schaudinn, A. Bethe, L. H. Wieler

14:00 – 15:30 Session 2: Pathogen-cell interaction (Room Steglitz)
Chairs: *Peter Valentin-Weigand and Jan Schinköthe*

14:00 **Efficient suilysin-mediated invasion and apoptosis in porcine respiratory epithelial cells after streptococcal infection under air-liquid interface conditions**
F. Meng, N.-H. Wu, M. Seitz, G. Herrler, P. Valentin-Weigand

14:15 **A GXXXA motif within the transmembrane domain of the Ebola virus glycoprotein is important for counteraction of the antiviral host factor tetherin**
M. González Hernández, M. Hoffmann, C. Brinkmann, S. Pöhlmann

14:30 **Recombinant Mumps viruses expressing the batMuV F protein are highly fusion active**
N. Krüger, C. Sauder, M. Hoffmann, C. Örvell, S. Rubin, G. Herrler

14:45 **A Neonatal Mouse Model To Study Invasive Non-Typhoidal *Salmonella* Typhimurium Infections: Insights Into The Role Of Salmonella Pathogenicity Island 2 (SPI2)**
K. van Vorst, A. Dupont, K. Zhang, C. Pfarrer, U. Repnik, G. Griffiths, M. Hensel, M. Hornef, M. Fulde

15:00 **Identification of molecular requirements for bat influenza A-like virus entry into mammalian cells**
M. Hoffmann, P. Zmora, N. Krüger, F. Wrensch, G. Herrler, S. Pöhlmann

15:15 **A comprehensive library of C-type lectin receptor (CLR)-Fc fusion proteins to detect CLR ligands on zoonotic pathogens**
J. Monteiro, S. Mayer, K. Opitz, R. Möller, T. Johannssen, **B. Lepenies**

14:00 – 15:30 **Session 3: Risk assessment, epidemiology and modelling**
(Room Zehlendorf)
Chairs: *Sandra Eßbauer and Martin Pfeffer*

14:00 **Distribution of the Usutu virus and its impact on bird populations in Germany**
R. Lühken, S. M. Thomas, N. Tjaden, H. Jöst, J. Knauer, U. Ziegler, M. Groschup, C. Beierkuhnlein, J. Schmidt-Chanasit

14:15 **Risk factors for human Campylobacter infections in Germany and source attribution**
B. Rosner, A. Schielke, X. Didelot, C. Josenhans, F. Kops, G. Gölz, T. Alter, K. Stingl, J. Breidenbach, S. Suerbaum, K. Stark

14:30 **Dynamic of excretion and immune response of experimentally infected pigs with monophasic variant of *Salmonella* Typhimurium serovar 1,4[5],12:i:-**
M. Cevallos, C. Houdayer, Y. Bailly, F. Paboeuf, C. Fablet, M. Denis, **A. Kerouanton**

14:45 **Modelling the risk of the establishment of *Aedes albopictus* in Germany using fine-resolution climate data**
A. Jaeschke, N. Tjaden, S. Thomas, C. Beierkuhnlein

15:00 **Risk factors for autochthonous Hepatitis E in Germany**
M. Faber, M. Askar, K. Stark

15:15 **Contact Networks in the pig barn – Finding MRSA in the noisy movement**
T. Kaufholz, M. Will, C. Müller-Graf, T. Selhorst

15:30 *Coffee Break and Poster Viewing*

16:00 – 17:30 **Big Data Management in Zoonoses Research (Room Ballsaal)**
Chairs: *Sebastian Semler and Anna Wendt*

16:00 – 16:20 **Management of Big (complex, federated, delicate…) Data in The National Cohort**
W. Hoffmann

16:20 – 16:40 **How to Manage Big Data in Zoonotic Research**
D. Harmsen

16:40 – 17:00 **Standardizing area-wide datasets from multiple sources, the case of mapping disease vectors**
G. Hendrickx

17:00 Discussion

17:30 – 19:30 **General Assembly German Research Platform for Zoonoses (Room: Ballsaal)**
Language: German
Chair*: Sebastian C. Semler*

19:30 ***Welcome Reception/Social Dinner***
Room: Ballsaal

Friday, October 14, 2016

07:30 – 09:00 **Young Scientists Breakfast**
Room: Restaurant

09:00 – 10:30 **Session 4: Antimicrobial use and resistance II (Room Ballsaal)**
Chairs: *Sebastian Guenther and Peter Klotz*

09:00 **Influx of extended-spectrum beta-lactamase—producing Enterobacteriaceae and methicillin resistant *Staphylococcus aureus* into an equine veterinary teaching hospital**
B. Walther, S. Klein, A.K. Barton, A. Lübke-Becker, H. Gehlen

09:15 **The RESET-research database: A comprehensive collection of microbiological and epidemiological information on ESBL-producing bacterial strains from Germany**
K. Hille, U. Seibt, W. Honscha, L. Kreienbrock

09:30 **Retrospective analysis addressing the emergence of the plasmid encoded colistin resistance gene mcr-1 in German livestock farms during the years 2011-2013**
N. Roschanski, M. Thieck, J. Hering, C. von Salviati-Claudius, H. Laube, M. Grobbel, L. Kreienbrock, **U. Rösler**

09:45 **Prevalence of extended spectrum and AmpC ß-lactamase producing Enterobacteriaceae in poultry during slaughter**
P. von Tippelskirch, G. Gölz, S. Orquera, M. Projahn, K. Dähre, A. Friese, U. Rösler, T. Alter

10:00 **Does size matter? - Transferability of extended-spectrum β-lactamase gene-**

carrying plasmids harboured by *Escherichia coli* isolates of diseased food-producing animals
G.B. Michael, A.K. Siqueira, K. Kadlec, H. Kaspar, S. Schwarz

10:15 **ESBL-/AmpC-producing Enterobacteriaceae in the broiler production chain**
K. Dähre, M. Projahn, A. Friese, U. Rösler

09:00 – 10:30 Session 5: Innate and Adaptive Immune Response
(Room Steglitz)
Chairs: *Veronika von Messling and Stephan Ludwig*

09:00 **Fruit bat tetherin restricts release of virus-like particles and is inefficiently antagonized by filovirus glycoproteins**
C. Brinkmann, I. Nehlmeier, M. Hoffmann, S. Pöhlmann

09:15 **An inactivated bivalent rabies - canine distemper virus vaccine induces protective immunity against both viruses in ferrets**
R. da F. Budaszewski, B. Sawatsky, B. Krämer, M. Schnell, V. von Messling

09:30 **Bat Mx proteins: evolution and antiviral specificity**
J. Fuchs, M. Hölzer, M. Schilling, C. Patzina, T. Hoenen, G. Zimmer, F. Weber, M. Marz, M.A. Müller, G. Kochs

09:45 **The immunopathogenic potential of *Arcobacter butzleri* – pathogen or commensal?**
M. M. Heimesaat, T. Alter, S. Bereswill, G. Gölz

10:00 **Correlating viral interferon antagonism with host evolutionary distance by a trans-species comparative interferon bioassay**
D. Ritz, J. Papies, D. Niemeyer, Andrea Sieberg1, Isabella Eckerle1, Anita Kretschmann, A. Pfeifer, F. Weber, C. Drosten, **M. A. Müller**

10:15 **Exploitation of PKR as a selector for the identification of immunostimulating influenza virus RNAs useful for antiviral therapy**
M. Budt, C. Mache, F. Jolmes, T. Wolff

09:00 – 10:30 Session 6: Novel methods, diagnostics and NGS
(Room Zehlendorf)
Chairs: *Katrin Kuhls and Martin Groschup*

09:00 **Automated whole genome sequence based serogrouping of *Listeria monocytogenes* isolates**
P. Hyden, A. Pietzka, A. Lennkh, A. Murer, B. Springer, A. Indra, D. Harmsen, F. Allerberger, C. W. Sensen, **W. Ruppitsch**

09:15 **Next-Generation Multiplex PCRs for Virus Diagnostics**
A. Brinkmann, A. Radonić, A. Nitsche

09:30 **Tracing the geographical origin of human brucellosis in Germany**
E. Georgi, B. H. Northoff, M.-T. Pfalzgraf, H.C. Scholz, L. M. Holdt, M. C. Walter, S. Zange, M. H. Antwerpen

09:45 **Revolutionizing outbreak investigations: a retrospective analysis of nosocomial LA-MRSA transmission in an Austrian hospital by next generation sequencing**
S. Lepuschitz, D. Schmid, B. Springer, A. Indra, F. Allerberger, W. Ruppitsch

10:00 **Crimean Congo Haemorrhagic Fever, 2014 Sudan**
C. Kohl, T. Muzeniek, M. Eldegail, I. Mahmoud, A. Brinkmann, P. W. Dabrowski, L. Schrick, A. Radonic, P. Emmerich, T. Rieger, S. Günther, A. Nitsche, A. A. Osman

10:15 **Microfluidically supported organoids for modelling systemic inflammation and infection**
A.S. Mosig, M. Gröger, K. Rennert, O. Huber

10:30 *Coffee Break and Poster Viewing*

11:00 – 12:30 Session 7: New and re-emerging zoonoses (Room Ballsaal)
Chairs: *Christian Drosten and Rainer Ulrich*

11:00 **In vivo screening for the novel zoonotic bornavirus (VSBV-1): New positive squirrel species and further sequences**
D. Hoffmann, K. Schlottau, C. Fast, D. Tappe, B. Hoffmann, D. Höper, C. Herden, R.G. Ulrich, M. Beer

11:15 **Link of a ubiquitous human coronavirus to dromedary camels**
I. Eckerle, V.M. Corman, Z. Memish, A.M. Liljander, R. Dijkman, H.R. Jonsdottiert, K. Juma Ngeiywah, E. Kamau, M. Younan, M. Masri, A. Assiri, I. Gluecks, B.E. Musa, B. Meyer, M.A. Müller, M. Hilali, S. Bornstein, U. Wernery, V. Thiel, J. Jores, J.F. Drexler, C. Drosten

11:30 ***Culex pipiens* and *Culex torrentium* mosquitoes from Germany are susceptible to infection with West Nile virus**
M. Leggewie, M. Badusche, M. Rudolf, S. Jansen, J. Börstler, R. Krumkamp, K. Huber, A. Krüger, J. Schmidt-Chanasit, E. Tannich, S. C. Becker

11:45 **Detection of Mycobacterium tuberculosis complex bacteria in feces, urine and saliva of red deer (*Cervus elaphus*) and cattle from the hotspot regions in Germany**
K. Schwaiger, T. Körner, S. Schex, S. Rupp, M. Gareis, A. Hafner-Marx, M. Müller, M. Büttner

12:00 **Discovery of novel bunyaviruses in mosquitoes from the neotropics**
A. Kopp, F. Zirkel, A. Hübner, D. Hobelsberger, A. Estrada, I. Jordan, T. R. Gillespie, C. Drosten, S. Junglen

12:15 **Poultry-associated multi-drug resistant *Salmonella* spp., *Campylobacter* spp. and *Arcobacter* spp. in urban Ghana**
D. Dekker, K. Boahen, D. Eibach, A. Zautner, W. Rabsch, N. Sarpong, J. May

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11:00 – 12:30 Session 8: Parasites
(Room Steglitz)
Chairs: *Anton Aebischer and Karsten Nöckler*

11:00 **The host cell carbohydrate metabolism regulates *Toxoplasma gondii* bradyzoite formation in skeletal muscle cells**
C. G. K. Lüder, T. Rahman, I. Swierzy, B. Downie, G. Salinas-Riester, M. Blume

11:15 **Epidemiology and genetic characterization of *Giardia* spp. infections in wild rodents in Germany**
C. Klotz, Y. Helmy, N. Kratzmann, S. Schmidt, U.M. Rosenfeld, S. Fischer, D. Reil, R.G. Ulrich, T. Aebischer

11:30 **Relationship between the seroprevalence in chicken and the presence or infectivity of *Toxoplasma gondii* cysts in chicken meat and other edible tissues**
G. Schares, B. Bangoura, M. Ludewig, F. Randau, A.-A. Alnassan, P. Maksimov, B. Matzkeit, M. Sens, A. Bärwald, F. J. Conraths

11:45 **Abundance of *Ixodes ricinus* and the prevalence of Lyme Borrelia pathogenic to humans**
A. Debski, F.-R. Matuschka, D. Richter

12:00 **Host-feeding patterns of mosquito species in Germany**
J. Börstler, H. Jöst, R. Garms, A. Krüger, E. Tannich, N. Becker, J. Schmidt-Chanasit, R. Lühken

12:15 **Comparison of different commercial DNA extraction kits to detect *Echinococcus multilocularis* eggs in faecal samples from foxes**
P. Maksimov, G. Schares, S. Press, A. Fröhlich, M. Herzig, F. J. Conraths

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11:00 – 12:30 Session 9: Free topics (Room Zehlendorf)
Chairs: *Martin Beer und Stephanie Thomas*

11:00 ***Mycobacterium avium* subspecies paratuberculosis in non-human primates**
K. Fechner, K. Mätz-Rensing, K. Lampe, F.-J. Kaup, C.-P. Czerny, J. Schäfer

11:15 **Co-Adaptation of the Hemagglutinin Due to Increased pH Stability is Essential in H5N1 HPAIV Evolution**
U. Wessels, E-S. M. Abdelwhab, J. Veits, O. Stech, T. C. Mettenleiter, **J. Stech**

11:30 **Two consecutive outbreaks of *Salmonella* Muenchen linked to pig farming in Germany 2013-2014: Is something missing in our regulatory framework?**
A. Schielke, W. Rabsch, R. Prager, S. Simon, A. Fruth, R. Helling, M. Schnabel, C.Siffczyk, S. Wieczorek, S. Schroeder, B. Ahrens, H. Oppermann, S. Pfeiffer, S.-S. Merbecks, B. Rosner, C. Frank, A. A. Weiser, P. Luber, A. Gilsdorf, K. Stark, D. Werber

11:45 **Joint use of data from science, public health institutions and industry partners: data structures, scientific aspects and legislative challenges in the context of animal health**
A. Wendt, D. Meemken, G. Klein, T. Blaha, K. N. Knöll, T. May, L. Kreienbrock

12:00 **SARS-CoV replication is regulated by p53 via interaction of the SARS-Unique Domain and PLpro with E3 ubiquitin ligase RCHY1**
A. von Brunn, J. Carbajo-Lozoya, M. Y. Hein, M. A. Müller, W. Deng, J. Lei, B. Meyer, Y. Kusov, B. von Brunn, D. R. Bairad, S. Hünten, C. Drosten, H. Hermeking, M. Mann, R. Hilgenfeld, Y. Ma-Lauer

12:15 **Cold-hardiness of the Asian tiger mosquito Aedes albopictus, a vector of multiple viral and parasitic pathogens**
R. Müller, A. Kreß, A.-M. Oppold, U. Kuch

12:30 *Lunch and Poster Viewing*

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

14:30 **Keynote 3:**
Ancient Pathogen Genomics: What we learn about Zoonosis from the past
Johannes Krause, Jena, Germany

15:15 **Keynote 4:**
Zika virus replication and vaccine
Pei-Yong Shi, Galveston, USA

16:00 **Poster Awards**

16:20 **Farewell**

General Information

Date and Venue
October 13-14, 2016
Best Western Plus Hotel Steglitz International
Albrechtstraße 2, 12165 Berlin
www.si-hotel.com

Conference Language
The official conference language is English.

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

Organization
Office of the German Research Platform for Zoonoses

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Friederike Jansen
Sebastian Sprengel

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Review Committee
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Peter Valentin-Weigand, Hannover, Germany
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Poster Award Committee
The poster awards for young scientists are selected by the members of the Internal Advisory Board of the German Research Platform for Zoonoses.

Keynote Speakers

Dirk Brockmann, Berlin, Germany
Johannes Krause, Jena, Germany
Ab Osterhaus, Hannover, Germany
Pei-Yong Shi, Galveston (TX), USA

Big Data Management in Zoonoses Research Session Speakers

Dag Harmsen, Münster, Germany
Guy Hendrickx, Zoersel, Belgium
Wolfgang Hoffmann, Greifswald, Germany

Young Scientists Breakfast
The Young Scientists Breakfast is going to take place at the “Pavillon” room of the hotel on Friday, October 14, at 7:30 am.
The attending senior scientists are:

Sascha Al-Dahouk, Berlin, Germany
Christa Ewers, Gießen, Germany
Alex Greenwood, Berlin, Germany
Ilse Jacobsen, Jena, Germany
Annika Schielke, Berlin, 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 2016 is registered for 6 CME points per day by the Berlin Chamber of Physicians (Ärztekammer Berlin). You will receive your certificate during the lunch breaks. 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 2016 is registered for 5 hours (ATF-Stunden) per day by the Federal Chamber of Veterinarians (Bundestierärztekammer). You will receive your certificate during the lunch breaks.

Poster Presentations
Posters will be presented during both days of the conference. Poster presenters should refer to this booklet 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.

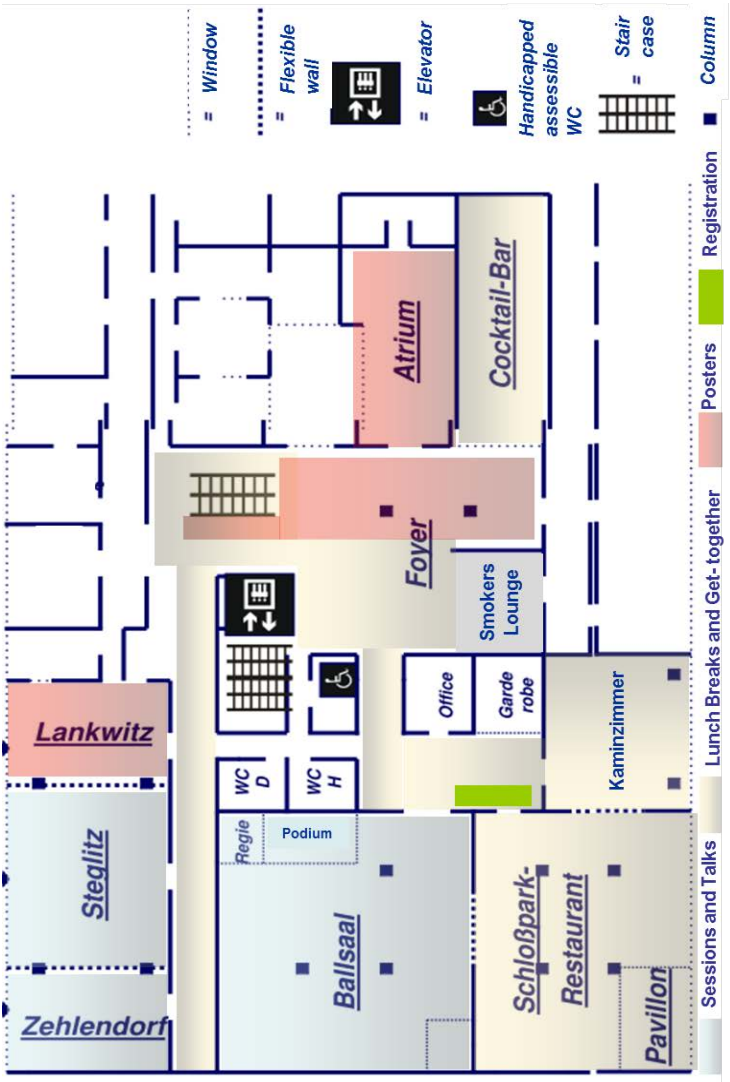
Oral Presentations
Oral presentations should be handed over on a common data carrier at the registration desk on Thursday, October 15, between 8.00 am and 1.00 pm. All session rooms will be equipped with a PC computer and a LCD projector. Apple computers are not available. Please make sure, that you use either a powerpoint or a pdf file format.

Internet Access
For internet access you are pleased to register at the hotel reception in the ground floor. WLAN will be provided without charge.

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

Sponsoring
We would like to thank the following sponsor:

Floor Plan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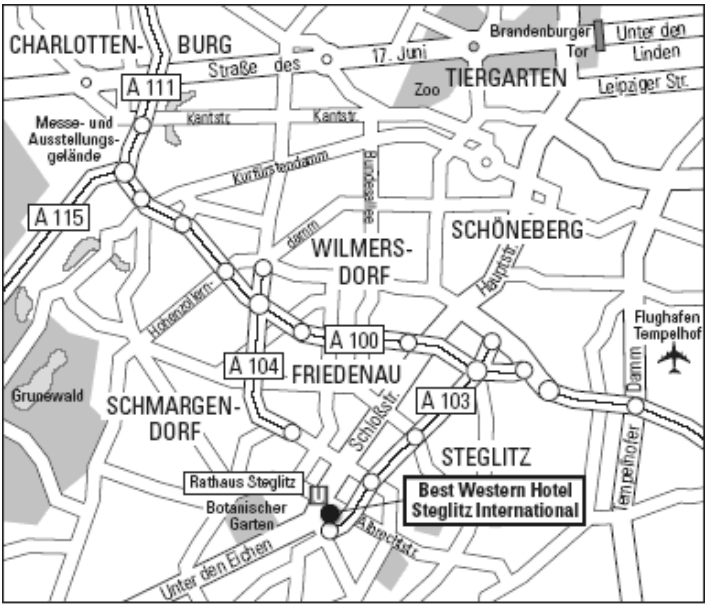
Pavillon

Site Plan


CHARLOTTEN-
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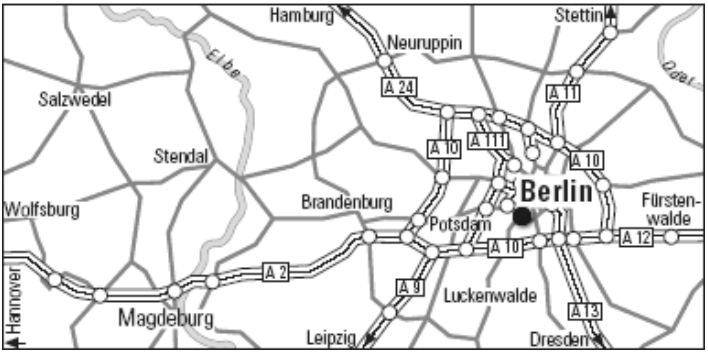
Straße des 17. Juni
Brandenburger Tor
Unter den Linden
TIERGARTEN
Zoo
Leipziger Str.
A 111
Messe- und Ausstellungs-gelände
Kantstr.
Kantstr.
Kurfürstendamm
A 115
WILMERS-DORF
SCHÖNEBERG
Hauptstr.
Hohenzollern-
A 100
A 104
FRIEDENAU
Flughafen Tempelhof
Grunewald
SCHMARGEN-DORF
A 103
Schloßstr.
Tempelhofer Damm
STEGLITZ
Rathaus Steglitz
Botanischer Garten
Best Western Hotel Steglitz International
Unter den Eichen
Albrechtstr.


Hamburg
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Leipzig
Dresden

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 700 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.

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.

Please feel free to contact the office if you have a workshop relevant for support and furtherance by the German Research Platform for Zoonoses.

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 13, 2016
10:30 – 12:00
Room: Ballsaal
Chair: Stephan Ludwig

and

October 14, 2016
14:30-16:00

Room: Ballsaal
Chair: Martin H. Groschup

Complexity, Networks and Disease Dynamics

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Keywords: Computational Epidemiology, Complex Networks, Complexity Science

The last decade has witnessed the emergence and global spread of new, often highly contagious and virulent pathogens that spread across the globe in a matter of weeks or months. Emergent infectious diseases have not only become a key threat to global public health, but carry the potential of yielding major economic crises. Understanding and predicting the geographic spread of emergent infectious diseases has become a major challenge to epidemiologists, public health organizations and policy makers. Large-scale computer simulations that integrate methods from statistical physics, complex network theory and dynamical systems theory have become a key tool in this context. I will report on state-of-the art research in this area and will focus on a recent theoretic approach that reveals hidden geometries in global contagion phenomena of today.

AIDS, Avian flu, SARS, MERS, Ebola, Zika …what next?

A. Osterhaus[1]

[1]'RIZ', Hannover, Germany and 'Artemis One Health' Utrecht, The Netherlands

Complex relationships between humans and animals have created an interface that allowed cross-species transmission, emergence and eventual evolution of a plethora of human pathogens. Until 1900, infectious diseases were the major cause of mortality of humankind, causing an estimated fifty percent of all deaths. In the western world, this decreased to only a few percent, due to the implementation of public health measures and the introduction of vaccines and antimicrobial compounds. This prompted policymakers and scientists to speculate that soon human infectious diseases would be brought under control. Paradoxically, soon thereafter the world was confronted with an ever-increasing number of emerging and re-emerging infectious diseases like AIDS, Avian flu, SARS, MERS, Ebola, and Zika, spilling over from animal reservoirs. A complex mix of predisposing factors in our globalizing world, linked to major changes in our societal environment and global ecology, collectively created opportunities for viruses and other pathogens to infect and adapt to new animal and/or human hosts. This paved the way for the unprecedented spread of infections in humans and animals with dramatic consequences for public and animal health, animal welfare, food supply, economies, and biodiversity. It is important to realize that due to the complex and largely interactive nature of the predisposing factors, it is virtually impossible to predict what the next pathogen threat will be, from where it will come and when it will strike. However, better understanding of the underlying processes may eventually lead to predictions that would improve our preparedness for outbreaks in humans and animals. Investment in a better understanding the human-animal interface will therefore offer a future head start in the never-ending battle against infectious diseases of humans. Importantly, the increased emergence of viral infections is largely paralleled by medical, veterinary, technological, and scientific progress, continuously spurred by our never-ending combat against pathogens. Especially the establishment of vaccine development platforms, widely applicable to both known and unknown viruses will further contribute to an R&D based response preparedness.

Ancient Pathogen Genomics: What we learn about Zoonosis from the past

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Keywords: human history, ancient pathogens, zoonosis, bacterial evolution

High throughput sequencing has revolutionized the field of archaeogenetics in the last decade, providing a better understanding of human history, past population dynamics and host pathogen interactions through time. Targeted DNA capture approaches have allowed reconstructing complete ancient bacterial genomes providing direct insights into the evolution and origin of some of the most infamous pathogens known to humans such as *Yersinia pestis*, *Mycobacterium tuberculosis* or *Mycobacterium leprae.* Here the potential of ancient pathogen genomics is discussed in the light of zoonosis research. Phylogenetic comparisons of modern and ancient bacterial genomes are presented that have provided direct evidence for zoonotic transmissions between humans and animals in the past that might have been responsible for the dissemination of *M. tuberculosis* in the Americas before European contact. Furthermore genome wide studies of *Y. pestis* over 5000 years of human history are discussed that provide evidence that major virulence factors essential for the transmission of bacteria by fleas have evolved rather late in human history. Temporal studies of pathogens might thus throw new light on the zoonotic origin of human diseases and potentially allow predicting and preventing further zoonotic transmissions in the future.

Zika virus replication and vaccine

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Keywords: Zika virus, flavivirus replication, vaccine

Background and objectives: The recent outbreaks of Zika virus have caused a huge public health burden. In this presentation, I will give an overview on Zika virus epidemiology, diseases, diagnostics, and vaccine and antiviral development. I will also present our work on the development of molecular tools to study Zika virus replication and to develop vaccine.
Materials and methods: Using an Asian lineage of Zika virus clinical strain, we have developed an infectious cDNA clone that could be used for mutagenesis studies. The mutant viruses were characterized in cell culture, mouse model, and mosquito vectors.
Results: An infectious cDNA clone has been established for Zika virus. The reverse genetic system could be reliably used to study viral replication. The mutant viruses showed various phenotypes in cell culture, mouse disease model, and mosquito transmission.
Conclusion: We have developed the reverse genetic system for Zika virus. Using the reverse genetic system, we have identified several key replication and virulent determinants that regulate Zika virus replication and pathogenesis. The attenuated Zika virus could be developed for potential vaccine.

Session 1: Antimicrobial use and resistance I

October 13, 2016
14:00 – 15:30

Room Ballsaal
Chairs: Birgit Walther and Hendrik Scheinemann

Whole-genome sequence analysis of ESBL-producing *E. coli* of ST410 from avian wildlife, human, companion animal and environmental origins indicates interspecies transmission

K. Schaufler[1], M. Wöhrmann[1], R. Baddam[1], K. Müller[2], P. Gastmeier[3], B. Kohn[2], T. Semmler[1,4], L. H. Wieler[1,4], C. Ewers[5], S. Guenther[1]

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Keywords: whole genome sequencing, ESBL-E. coli, transmission

Background and Objectives: The occurrence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* was initially restricted to a human and veterinary clinical context, but recent findings prove their prevalence in extra-clinical settings such as the environment and wildlife. We comparatively analysed ESBL-producing *E. coli* using whole-genome sequence (WGS) data to get insights into their possible transmission between different habitats.
Material and Methods: A total of 491 *E. coli* isolates from environmental (wild birds, dog faeces) and clinical origin (human, canine) were screened for the occurrence of ESBL-producing *E. coli*. A selection of isolates based on MLST and PFGE was whole-genome sequenced and WGS data of ten isolates of ESBL-producing *E. coli* of ST410 from different habitats was used for single nucleotide polymorphism (SNP) and PLACNET analysis.
Results: Irrespective of their origin, our analysis revealed a very high genetic similarity for the ten ST410 ESBL-producing *E. coli* isolates, differing by low numbers of SNPs only. Similar resistance patterns, serotypes and plasmid profiles reinforce their clonal character.
Conclusions: Our results underline the mandatory nature of the “One Health” approach as we have initial evidence for a recent interspecies transmission of a new successful and zoonotic clone of ST410 *E. coli* between wildlife, humans, companion animals, and the environment.

Cattle as a source of *Acinetobacter baumannii* and Carbapenemase-positive *Acinetobacter indicus*

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Keywords: Carbapenem resistance, Acinetobacter, cattle

Background and objectives: Infections with *Acinetobacter baumannii*, particularly those with resistance to carbapenems, are an emerging public health issue. While there is evidence for the emergence of *A. baumannii* in pets, their presence in livestock is largely unexplored.
Materials and methods: 410 cattle (nasal/rectal swabs, composite fecal samples) from 353 farms in Hesse were screened for *Acinetobacter* spp.. Species were identified by MALDI-TOF MS, *gyr*B-PCR and 16S rRNA sequencing. Antimicrobial susceptibility was tested with the VITEK2-system. PCRs were used to identify β-lactamase genes ($bla_{\text{OXA-23/24/58}}$) and assign international clones (IC). Copy strains were identified with PFGE.
Results: 63 cattle (15.4%) carried *A. baumannii*, mainly in the nasal cavity, all of them being susceptible to carbapenems. 17 isolates grouped within IC2, 14 within IC3, the remaining isolates were non-IC1-3 strains. PFGE revealed that cattle carried 1 to 3 different *A. baumannii* strains. *A. indicus* was isolated from 18 cattle; 6 and 2 isolates carried $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$, respectively; one isolate showed high MICs to imipenem (8 µg/ml).
Conclusion: *A. baumannii* of IC1 are frequently associated with human infections and our data suggest that cattle might be a source of such strains. The frequent finding of carbapenemase-positive *A. indicus* requires further investigations on genomic characteristics, antimicrobial resistance and zoonotic relevance of this new *Acinetobacter* sp., which for the first time has been isolated from a human patient in 2014.

Antibiotic usage in livestock in Germany - Which drug classes are used?

S. Kasabova[1], M. Hemme[1], L. van Rennings[1], M. Hartmann[1], I. Ruddat[1], C. von Münchhausen[1], A. Kaesbohrer[2], L. Kreienbrock[1]

[1]University of Veterinary Medicine Hannover; [2]Federal Institute for Risk Assessment, Berlin

Keywords: antibiotic use, monitoring, treatment frequency

Within the research project VetCAb (Veterinary Consumption of Antibiotics) data about the use of antibiotics in livestock in Germany in the last several years were collected and evaluated. The aim of the study was to analyse how often food producing animals were treated with antibiotics during a given time period. Longitudinal data of the results were already presented at the National Symposium on Zoonoses Research last year. These results showed that the treatment frequency is on the decrease in many animal holdings.
Against the background of these evaluations the question is raised – is the decrease of the treatment frequency based on actual minimization of the consumption or mainly on shifting of prescriptions to other drug classes.
Data for pigs from over 450 pig holdings in Germany are going to be evaluated and presented. Currently over 50000 records of the years 2011, 2013, 2014 and 2015 are available in the database, providing the basis for this evaluation. The treatment frequency per drug class is going to be calculated as a percentage of the total treatment frequency to observe if, and if so, how the percentage per drug class is changing over the last years.
Considering the global resistance situation particular attention should be paid to treatment frequencies of antibiotic classes, classified from the World Health Organisation as critically important such as quinolones, 3rd and 4th generation cephalosporins and macrolides, which are also approved for food producing animals. The percentage distribution of treatment frequency in pigs by age group and antibiotic class is going to be the focus of this presentation, with an emphasis on movements in the usage of antimicrobials, their possible reasons and consequences.

Disrupting the fatal interplay of influenza virus and pneumococcal neuraminidases by dual-acting inhibitors

A. Hoffmann[1], M. Richter[1], E. Walther[1], Z. Xu[1], L. Schumann[1], U. Grienke[2], C.E. Mair[2], J. Kirchmair[3], S. von Grafenstein[4], K.R. Liedl[4], V. Makarov[5], S. Nietzsche[6], W. Pfister[7], J.M. Rollinger[2], M. Schmidtke[1]

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Keywords: influenza virus, Streptococcus pneumoniae, neuraminidase

The interplay of neuraminidases (NAs) of influenza virus and *Streptococcus pneumoniae* contributes to disease severity in co-infected influenza patients. Dual acting NA inhibitors (NAIs) might be able to disrupt this synergism.

We selected potential NAIs by phenomenological search and virtual screening. Target-based assays were used to identify NAIs acting on viral and pneumococcal NAs. Their binding mode was studied by enzyme kinetics, reverse genetics, and recombinant pneumococcal NAs. Antiviral and antibacterial activities were determined by measuring the inhibition of viral cytopathic effect, bacterial growth and biofilm formation. Additionally, we showed the inhibitory effect of NAIs on viral replication in the presence of pneumococcal NA.

We identified dual acting NAIs from two compound classes, prenylated flavonoids and azo compounds, both showing stronger activity on bacterial NAs. Mixed type of inhibition was confirmed for azo compounds. In agreement with this, amino acid substitutions in the NA active site did not severely abrogate NAI activity. Some NAIs exerted antiviral and antibacterial activity. Whereas the prenylated flavonoids act bactericidal, bacteriostatic activity was detected for azo compounds.

While further mechanisms underlying the observed bioactivity cannot be ruled out, these results warrant *in vivo* studies for proving the therapeutic effect of dual acting NAIs on lethal synergism between influenza virus and pneumococci.

Harmonisation of biocide susceptibility testing of bacteria

A. T. Feßler[1], F. Geber[2], M. Meurer[1], V. Hensel[1], S. Speck[2], M. Reinhardt[2], G. B. Michael[1], U. Truyen[2], S. Schwarz[1]

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Keywords: broth macrodilution, disinfectants, biocide tolerance

Background and objectives: The aim of this study was to develop a harmonised method for biocide susceptibility testing.
Materials and methods: Tests were performed for *Staphylococcus aureus* ATCC® 6538 with benzalkonium chloride (BAC), chlorhexidine (CHX) (2-fold dilutions) and isopropyl alcohol (ISO) (3 % steps), comparing different conditions such as volumes (2 mL and 10 mL), subcultures, inoculum preparation methods and incubation times (24 h, 48 h and 72 h). The biocide dilutions were prepared in half of the final volume and mixed with the same amount of double concentrated tryptic soy broth. The tubes were inoculated with 1 x 10^5 - 1 x 10^6 cfu/mL test volume.
Results: In total, 144 MIC values per biocide were obtained and revealed 0.0001 % for BAC (n=82/144) and CHX (n=119/144) and 7 % for ISO (n=104/144). Deviations of more than +/- one dilution step were only seen once for BAC and for ISO after 72 h (due to the evaporation of the ISO which could be avoided by additional sealing).
The proposed protocol for biocide susceptibility testing is as follows: Two-fold-dilution series are used for concentrations ≤ 1 % and 2 %-steps for concentrations > 1 % in 2 mL volumes. A fresh overnight culture is used and the inoculum can be prepared either by direct colony suspension or the use of glass beads and incubation is performed at 37°C for 24 h.
Conclusion: This protocol shall contribute to a harmonisation of the biocide susceptibility testing of bacteria.

Characterization of the phenotypic antimicrobial resistance and biofilm profile of mastitis-associated bacteria

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Keywords: Mastitis, biofilm, minimal inhibitory concentration (MIC)

Background and objectives:
Mastitis is a worldwide cause of enormous financial losses in dairy industry. The general therapeutical approach is the use of antibiotics, which is partly ineffective if the inflammation is caused by a biofilm forming pathogen.
Materials and methods:
Seven mastitis-relevant bacterial pathogens were defined and 50 isolates per bacteria MALDI-TOF MS and MICs were determined using MICRONAUT-S Mastitis 3 plates (MERLIN diagnostics).
Per species, a subset of 8 isolates was chosen for biofilm experiment.
Results:
To date, this study includes 438 isolates of clinical changed mammary gland of cows.
MIC testing revealed a rather low variability between the strains and a low rate of multidrug resistant (MDR) bacteria. We were able to identify one MDR *E. coli* and one Methicillin-resistant *S. aureus* (MRSA).
Most of the isolates were identified as good biofilm formers (CFU $1{,}0x10^5$-$1{,}0x10^7$/5 mm^2) compared to an EAEC known to be an excellent biofilm former (CFU $1{,}0x10^6$/5 mm^2). Lower CFU were determined within the tested streptococci (CFU 0-$1{,}0x10^5$/5 mm^2).
Conclusion:
The heterogenic species collection showed quite sensitive resistance patterns consistent with the literature and routine diagnostic. The majority of the mastitis associated isolates were able to form a stable biofilm, which has to be considered for a successful treatment and prophylactic approaches.

Session 2: Pathogen-cell interaction

October 13, 2016
14:00 – 15:30

Room Steglitz
Chairs: Peter Valentin-Weigand and Jan Schinköthe

Efficient suilysin-mediated invasion and apoptosis in porcine respiratory epithelial cells after streptococcal infection under air-liquid interface conditions

F. Meng[1], B. N.-H. Wu[1], M. Seitz[2], G. Herrler[1], P. Valentin-Weigand[2]

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Keywords: Streptococcus suis, apoptosis, air-liqiud interface culture

Background and objectives: Streptococci may colonize the epithelium in the airways and other entry sites. While local infection often remains asymptomatic, severe or even fatal diseases occur when streptococci become invasive and spread to different sites in the infected host. As representative of the streptococcal family we chose *Streptococcus suis* (*S. suis*) that is not only a major swine respiratory pathogen but occasionally also infects humans.
Materials and methods: We have established porcine respiratory air-liquid interface cultures (ALI) from the porcine lung to analyze the interaction of streptococci with their primary target cells. The airway epithelial cells were infected from apical compartment by *S. suis*.
Results: By comparing an *S. suis* wt strain with a suilysin-deficient mutant (10Δsly), we demonstrate that suilysin contributes to (i) adherence to airway cells (ii) loss of ciliated cells (iii) apoptosis, and (iv) invasion. A striking result of our analysis was the high efficiency of *S. suis*-induced apoptosis and invasion upon infection under ALI conditions.
Conclusion: We conclude that soluble effectors such as suilysin are present at higher concentrations in cells kept at ALI conditions and thus more effective. These results should be relevant also for infection of the respiratory tract by other respiratory pathogens.

A GXXXA motif within the transmembrane domain of the Ebola virus glycoprotein is important for counteraction of the antiviral host factor tetherin

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Keywords: Ebola virus, glycoprotein, tetherin

Ebola viruses (EBOV) cause severe disease in humans and non-human primates and the recent Ebola virus disease epidemic in West Africa highlighted that EBOV poses a global health threat. The glycoprotein (GP) is the sole viral surface protein and mediates host cell entry, counteracts the antiviral factor tetherin during viral release and causes cellular detachment, which may contribute to viral pathogenesis. It has been shown that a GXXXA motif within the transmembrane domain (TD) of GP is required for cellular detachment. Here, we investigated whether the GXXXA motif is required for other GP functions.

Using PCR-based mutagenesis the GXXXA motif was changed to LXXXL and virus-like particles and rhabdoviral vectors were used to analyse the importance of the GXXXA motif for tetherin counteraction and GP-mediated entry.

We observed that mutation of the GXXXA motif does not impact GP expression and proteolytic cleavage by furin and slightly reduces entry into mammalian or bat cell lines. However, disruption of the GXXXA motif largely abrogated tetherin counteraction by GP and thus affords the opportunity to determine if tetherin counteraction is essential for viral spread in the host. As a first step, we are currently analysing if this motif is required for spread of VSV encoding EBOV-GP in tetherin-positive cell lines and primary tissues.

Our results indicate that an intact GXXXA motif within the TD of EBOV-GP is dispensable for GP expression but required for tetherin counteraction.

Recombinant Mumps viruses expressing the batMuV F protein are highly fusion active

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Keywords: bat-derived paramyxovirus, mumps virus, fusion

A recent study reported the detection of a bat-derived virus (BatPV/Epo_spe/AR1/DCR/2009, batMuV) with a high phylogenetic relatedness to human mumps viruses (hMuV), raising the question if bats may serve as an intermediate or reservoir host for MuV. Since efforts to isolate infectious batMuV have reportedly failed, the evaluation of a possible interspecies transmission of batMuV is challenging.

We generated recombinant mumps viruses (rMuV) in which the ORFs for the fusion (F) and hemagglutinin-neuraminidase (HN) glycoproteins of a hMuV strain were replaced by the corresponding ORFs of batMuV.

The batMuV glycoproteins were incorporated into rMuV and the resultant virus was able to infect target cells. Differences were observed between the fusogenicity of rMuVs expressing one or both batMuV proteins. Viruses expressing batMuV F were highly fusogenic, regardless the origin of the HN. In contrast, rMuVs expressing hMuV F and batMuV HN were less fusogenic compared to hMuV. While the growth kinetics of rMuVs expressing batMuV HN together with hMuV or batMuV F were similar to the backbone virus, a lower starting virus titer was determined for rMuVs expressing batMuV F and hMuV HN. Neutralizing antibodies inhibited infection mediated by all rMuVs generated. The enzymatic activity of batMuV HN was similar to that of hMuV.

Our study reports the generation of chimeric MuVs expressing the F and HN proteins of batMuV, providing a means for further examination of this novel bat virus.

A Neonatal Mouse Model To Study Invasive Non-Typhoidal *Salmonella* Typhimurium Infections: Insights Into The Role Of Salmonella Pathogenicity Island 2 (SPI2)

K. van Vorst[1], A. Dupont[2], K. Zhang[2], C. Pfarrer[3], U. Repnik[4], G. Griffiths[4], M. Hensel[5], M. Hornef[2], M. Fulde[1]

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Keywords: Salmonella Typhimurium, Salmonella Pathogenicity Island 2, Salmonella-containing vacuole

Non-typhoidal Salmonella are among the most prevalent causative agents of infectious diarrheal disease in humans and pigs and contribute to invasive infections in human infants. The pathogenicity of *Salmonella* is conferred by horizontally acquired chromosomal regions, called Salmonella pathogenicity islands (SPIs), encoding specific type-three secretion systems and sets of effector proteins. While SPI1 facilitates bacterial uptake into non-phagocytic cells, SPI2 is crucial for the intracellular establishment of the *Salmonella* containing vacuole (SCV), allowing intracellular survival and replication [1,2]. We used our newly established neonatal mouse model [3] to study the contribution of SPI2 to the establishment and progression of systemic *Salmonella* infections.

Oral infection of neonatal mice with wildtype and SPI2-deficient *Salmonella* resulted in similar bacterial loads of the gastrointestinal tract, but decreased colony counts in systemic organs. In contrast to the general understanding of SPI2 as prerequisite for SCV formation, mutants were able to establish and maintain SCVs. In fact, mutants grow to high numbers inside SCVs without harming their host cell. By evaluating single SPI2 effector knock-out strains, we demonstrate that SifA, which is anchored to the SCV´s membrane, contributes to the SPI2-dependent phenotype. Our results suggest that its lack prevents SCV transmigration of neonate enterocytes and systemic spread *in vivo*.

Identification of molecular requirements for bat influenza A-like virus entry into mammalian cells

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Keywords: bat Influenza A-like virus, entry, host cell factors

Influenza A viruses (FLUAV) are enveloped, negative-stranded RNA viruses that pose a major threat to public health. Until recently, waterfowl was proclaimed the sole natural reservoir, but with the detection of FLUAV-related genomic sequences in bats (batFLUAV), this dogma seems to change. Host cell entry of FLUAV is orchestrated by the viral hemagglutinin (HA) that binds to sialic acids and mediates fusion of viral and cellular membranes. Although batFLUAV possess HA-like (HAL) proteins their function in host cell entry is incompletely understood.

To functionally characterize batFLUAV HAL in the absence of a virus isolate and to determine whether host cell factors promoting batFLUAV entry are present in human and other mammalian cells, we employed rhabdoviral pseudotypes.

Vectors equipped with HAL were able to enter predominantly bat cell lines but also one human and two canine cell lines were susceptible. Host cell entry driven by HAL was dependent on prior proteolytic activation and endosomal low pH. In contrast, sialic acids were dispensable for HAL-driven entry. Finally, type II transmembrane serine proteases (TTSPs) were able to activate HAL for cell entry, indicating that batFLUAV can readily utilize human proteases for HAL activation.

Collectively, our results identify viral and cellular factors governing cellular entry driven by batFLUAV surface proteins and indicate that at the stage of host cell entry the species barrier of batFLUAV is low.

A comprehensive library of C-type lectin receptor (CLR)-Fc fusion proteins to detect CLR ligands on zoonotic pathogens

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Keywords: Innate immunity, C-type lectins, dendritic cells

Background and objectives: The innate immune system provides a first line of defense by recognition of conserved microbial patterns on pathogens. C-type lectin receptors (CLRs) are carbohydrate-binding proteins that are predominantly expressed by antigen presenting cells. A common feature of CLRs is the presence of a carbohydrate recognition domain (CRD) that mediates Ca^{2+}-dependent binding of glycan structures. CLR/glycan interactions contribute to the initiation of innate responses including phagocytosis, cytokine production, and enhanced antigen presentation to T cells.
Materials and methods: To enable a high-throughput screening for CLR/pathogen interactions, we have generated a comprehensive library of murine and human CLR-Fc fusion proteins. To this end, the extracellular part of the respective CLR containing the CRD was fused to the Fc fragment of human IgG1 molecules. Currently, the CLR-Fc library covers the Dectin-1 and Dendritic cell immunoreceptor (DCIR) family and allows for pathogen binding studies by ELISA-based methods, flow cytometry as well as lectin array.
Results: Using the established CLR-Fc library, we found several novel CLR/pathogen interactions. Among others, the CLR Mincle was identified as a novel receptor for *Streptococcus spp.*
Conclusion: A comprehensive CLR-Fc fusion protein library containing more than 20 immunologically relevant CLRs is now available as a screening platform. It will enable to unravel yet unknown CLR ligands present on zoonotic pathogens.

Session 3: Risk assessment, epidemiology and modelling

October 13, 2016
14:00 – 15:30

Room Steglitz
Chairs: Sandra Eßbauer and Martin Pfeffer

Distribution of the Usutu virus and its impact on bird populations in Germany

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Keywords: Usutu virus, bird population trends

Background and objectives: The Usutu virus (USUV) is a mosquito-borne virus, circulating in an enzootic transmission cycle with birds as amplifying hosts and ornithophilic mosquitoes as vectors. In humans, USUV is generally expected to cause a mild arboviral disease, but also meningoencephalitis was observed in immunocompromised patients. USUV outbreaks with massive bird die-offs were recorded in several European countries (e.g. Italy, Austria). However, the population-level impacts of USUV in wild birds are largely unknown.
Materials and methods: Data on dead bird surveillance in combination with bioclimatic data were used to predict the potential distribution of USUV in Germany. Population data of 11 widespread bird species (e.g. Common blackbird, House sparrow) were compared between areas suitable and unsuitable for USUV.
Results: The highest probability of USUV occurrence was identified for south-west Germany covering approximately 5% of the entire country. In the comparison between the areas suitable and unsuitable for USUV, only the Common blackbird showed significant differences in the breeding bird population trends. The population of the species significantly declined in the USUV suitable areas with a total population reduction of approximately 25% between 2010 and 2013.
Conclusion: The analyses of the potential distribution of USUV indicated the highest risk for USUV transmission in the south-west of Germany, representing a risk for animal and public health.

Risk factors for human *Campylobacter* infections in Germany and source attribution

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Keywords: Campylobacter, risk factors, source attribution (max. 3)

Background and objectives:
Campylobacter infection is the most commonly reported bacterial gastroenteritis in Germany. We performed a case control study to investigate risk factors for *Campylobacter* infections. A source attribution analysis based on molecular strain typing (MLST) was conducted to determine routes of transmission.
Materials and methods: Case patients and randomly selected controls completed a questionnaire (study period Nov 2011-Feb 2014). *Campylobacter* isolates of a subset of case patients were further analyzed by MLST. We conducted logistic regression analyses to determine risk factors. Source attribution analysis was performed using an asymmetric island model.
Results: Consumption of chicken meat; preparation of poultry meat in the household; preparation of non-heated food together with raw meat; eating out; contact with poultry animals; and the use of gastric acid inhibitors were identified as risk factors for *Campylobacter* infections. As risk factors for *C. coli* infections we identified consumption of pork and use of gastric acid inhibitors. The majority of human *C. jejuni* infections were attributed to chicken, whereas for *C. coli* infections the main sources were chicken and pig.

Dynamic of excretion and immune response of experimentally infected pigs with monophasic variant of *Salmonella* Typhimurium serovar 1,4[5],12:i:-

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Keywords: monophasic Salmonella Typhimurium, pigs, excretion

In the last years, monophasic variant of *Salmonella* Typhimurium was highly prevalent in human salmonellosis. This serovar is also one of the most dominant in pigs. We followed the dynamic of excretion and the immune response of pigs infected by this serovar through an experimental trial.

8 SPF piglets of 7 week old were orally inoculated with 10^8 UFC of a monophasic *S.* Typhimurium strain. Then during 12 weeks, individual feces and blood were collected twice or once a week respectively. At the end of the trial, tonsils, mesenteric ganglions, and content of duodenum, jejunum, ileum and caeca were sampled. S*almonella* were numerated in UFC/g and Swine *Salmonella* antibodies in serum were dosed in %OD using IDDEX ELISA Kit.

Pigs excreted *Salmonella* just after inoculation and remain positive during all the trial. The level of excretion varied from 1 to 4.4 $\log_{10}$ CFU/g of feces, and fluctuates over time. *Salmonella* was present in all type of samples from autopsies except in ganglions. Tonsils were highly contaminated. *Salmonella* antibodies were present for two pigs within one week and for all pigs 6 weeks after inoculation. No correlation was observed between the quantity of *Salmonella* present in feces and the level of *Salmonella* antibodies.

This study allowed us to see the ability of this serovar to persist a long time in pigs after infection. Although individual carriage was variable, the persistence of the bacteria in the pig group is certainly due to contamination between animals.

Modelling the risk of the establishment of *Aedes albopictus* in Germany using fine-resolution climate data

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Keywords: climate change, species distribution model, vector-borne disease

Background and objectives: The Asian tiger mosquito *Aedes albopictus* is a competent vector of more than 20 diseases. After its introduction in Germany, climatic conditions need to be fulfilled for the successful establishment of the mosquito. With ongoing climate change, areas at risk of vector establishment need to be identified to support monitoring and surveillance measures.
Materials and methods: We applied an ensemble of correlative species distribution models to assess the current and near future climatic suitability for *Ae. albopictus* in Germany. For the first time, models were fitted using European occurrence records and bioclimatic variables taken from Worldclim (2.5 arcmin resolution). Model projections for Germany were done with Euro-LST satellite data using a 250 m resolution.
Results: Mainly, three areas within Germany could be identified as being currently climatically suitable: parts of Baden-Württemberg, Saarland and North Rhine-Westphalia. Based on the fine-scale resolution even more details, e.g. cities, hot spots, can be identified. Future projections indicate increasing suitability in Germany within the next years. With increasing suitable areas, the establishment of the vector becomes more likely in the near future.
Conclusion: With fine-resolution climate data current and future areas at risk are highlighted in high precision. Based on this information, areas for monitoring and surveillance can be identified.

Risk factors for autochthonous Hepatitis E in Germany

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Keywords: hepatitis E, epidemiology, risk factors, zoonosis, foodborne infections

Background and objectives: In Germany, Hepatitis E is a foodborne infection, mainly transmitted via consumption of pork and game meat and causing >1200 notified clinical cases in 2015. The objective of this study was to assess risk factors for autochthonous hepatitis E in Germany.
Materials and methods: We conducted a case-control study using symptomatic hepatitis E cases reported to local health departments between 01/2012 and 01/2014. Random digit dialling was used to recruit controls individually matched on sex, age group and area of living (4:1). Demographic, clinical and exposure data within two months before disease onset were collected in semi-standardized telephone interviews. Univariable and stepwise conditional logistic regression analysis (cutoff: $p<0.05$) was used to calculate matched odds ratios (mOR).
Results: In total, 270 cases and 1168 controls were included in the analysis (mean age 53 years and 61% men in both groups). Exposures associated with disease in the final model were: consumption of undercooked pork liver, pork meat and wild boar meat, frankfurters and liver sausage, raw vegetables and contact to waste water (occupational) [all mORs between 1.9 and 5.5, p-values<0.03]. Various host factors also remained significant.
Conclusion: Consumption of various pork products appears to be the main risk factor for autochthonous hepatitis E in Germany but host factors may greatly modify this risk. The role of boiled sausages and raw vegetables needs further research.

Contact Networks in the pig barn – Finding MRSA in the noisy movement

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Keywords: MRSA, Contact-Networks, Modelling

Contact networks are important to understand the epidemiology of any given infectious disease. Research into the social structure of livestock is still limited, in spite of the major role zoonotic pathogens play in public health.
We will – for the first time – analyse the contact structure between pigs in a barn during ongoing production. This work is done within the BMBF research project MedVetStaph in cooperation with the „Lehr- und Versuchszentrum Futterkamp der Landwirtschaftskammer Schleswig-Holstein".
Measuring contacts between animals is especially challenging. As opposed to humans where most interaction is face-to-face, pigs interact physically in various ways. We therefore decided to measure contact indirectly through the position of individual animals. To do this we have marked about 200 sows with a location tracking device.
Localisation mainly depends on the frequency of the measurement and the size of the area. In our case we have a temporal resolution of 1Hz and a barn of 600sqm. In this context, the relevant factor for defining a contact is determined by the error of the position tracking measurement and not only the actual movement of the animal. Therefore, it is essential to generate a robust contact network in spite of the uncertainties in the tracking process.
To assess this uncertainty and the impact it has on the contact threshold, we have modelled in this preliminary study the movement of the animals based on the situation at our research site.

Session: Management of Big Data in Zoonoses Research

October 13, 2016
16:00 – 17:30

Room Ballsaal
Chairs: Sebastian Semler and Anna Wendt

Management of Big (complex, federated, delicate,...) Data in The National Cohort

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Keywords: prospective cohort, comprehensive health assessment, whole-body MRI, biobanking

The German National Cohort (NAKO) is a cooperative multicentre prospective cohort study conducted by scientists from Universities, the Helmholtz and the Leibniz Associations, and other research institutes. NAKO investigates etiologic risk factors and preclinical changes in the pathogenesis of major chronic diseases including cardiovascular diseases, cancers, diabetes, psychiatric, neurodegenerative, respiratory, musculoskeletal, and infectious diseases, to generate evidence for primary and secondary prevention. Across Germany, a population registry based stratified representative random sample of 100,000 women and 100,000 men aged 20-69 years is being invited to 18 regional study centres. The baseline assessments include an extensive interview and self-completion touch screen questionnaires, an extended array of medical examinations and the collection of various biomaterials. A random subgroup of 20% of the participants (N = 40,000) receives an intensified examination ("Level 2") programme. Some 30,000 study participants undergo whole body magnetic resonance imaging. After 4-5 years, all participants will be invited for a re-assessment.
Chronic disease endpoints will be ascertained in active follow-up including, where possible, record linkage, e.g. with cancer registries. A comprehensive central data management ensures data privacy, online monitoring and reporting, and the implementation of a fair and transparent use and access policy for data and biomaterials.

How to Manage Big Data in Zoonotic Research

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Keywords: WGS, genomics, nomenclature, big data

Background: Whole genome sequencing (WGS) is on the verge of revolutionizing our ability to investigate and control outbreaks and understand transmission patterns – all with increased timeliness and accuracy. However, when setting up WGS projects usually the substantial costs for non-production activities such as comprehensive communication of comparable results, development and usage of bioinformatics tools, and big data storage - are ignored. To tackle part of those problems a European vision paper that was endorsed following the 2011 EHEC crisis states that as a number one priority, 'a common nomenclature ... for molecular typing data validation should be ensured where appropriate for human, food, and animal isolates so that any molecular typing data produced is comparable'.
Results: We have developed in the framework of the EU PathoNGenTrace project the genome-wide core genome MLST (cgMLST) gene allele calling approach that delivers a microbial genomic nomenclature that can be used to easily communicate comparable results. The associated nomenclature is hosted on public and federated servers that are tightly connected with public raw read archives such as NCBI SRA. More recently the U.S. CDC adopted and is currently implementing the cgMLST approach for its PulseNet USA and International networks to enable for a national and supra-national genomic real-time surveillance of food-borne zoonotic pathogens.
Conclusion: By using a genomic nomenclature big data are tremendously reduced in size and complexity.

Standardizing area-wide datasets from multiple sources, the case of mapping disease vectors

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Keywords: Area-wide Data Sets, Data sharing, Data quality

Emerging diseases in general and vector-borne diseases in particular are an increasing public and veterinary health concern, raising the need to map the distribution and abundance of potential vectors of disease. Existing information is scattered, formats can differ widely and many geographical gaps remain. Therefore, methods and tools have to be developed to collect, validate, standardise and optimise such data. Two recent examples highlight how this can be achieved: VectorNet and VECMAP.

VectorNet is a European network, funded by ECDC and EFSA and coordinated by Avia-GIS, for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents in Europe. To achieve this a web-based tool was developed and embedded in the ECDC information system including making map outputs available through the E3 geoportal.

VECMAP is a one-stop-shop for the area-wide mapping and spatial modelling of vector distribution and abundance, developed by Avia-GIS and its partners. It is designed for use by experts in fields such as entomology, biodiversity, epidemiology, forestry and agriculture. But it does not require expert knowledge of remote sensing or spatial modelling to use. The system includes the option to strengthen model outputs trough the secured sharing of information between VECMAP community members.

In this presentation we discuss how these tools are complementary to achieve the common goal where data quality is more important than data quantity.

Session 4: Antimicrobial use and resistance II

October 14, 2016
09:00 – 10:30

Room Ballsaal
Chairs: Sebastian Guenther and Peter Klotz

Influx of extended-spectrum beta-lactamase—producing Enterobacteriaceae and methicillin resistant *Staphylococcus aureus* into an equine veterinary teaching hospital

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Keywords: MRSA, ESBL-producing Enterobaceriaceae, horse clinic

Background and objectives: The influx rate of multi-drug resistant (MDR) pathogens including methicillin resistant *Stapyhlococcus aureus* (MRSA), *Acinetobacter baumannii* and extended-spectrum beta-lactamase(ESBL)-producing Enterobactericeae via colonized equine patients in clinical settings is worth knowing, since colonized patients provide a continuing source of MDR pathogens for themselves, other patients and not least the personnel and the environment.
Materials and methods: A total of 341 equine patients were screened for MDR pathogen-carriage. Horses suffering from either colic (n=233) or open wounds (n=108) were selected for microbiological examination of nostril swabs and fecal samples directly at hospital admission.
Results: Altogether 9.6% (31/323) of the valid fecal specimens were positive for ESBL-producing Entrobacteriaceae (94% *E. coli*), while MRSA (0.6%) and *A. baumannii* (0.9%) were rarely detected.
Results from the nostril swabs of both equine patient groups revealed an overall carriage rate of 3.5% for MRSA (12/340), ranging from 1.9% (wounds: 2/108) to 4.3% (colic: 10/232). Likewise, the ESBL-producing Enterobactericeae rate was 3.4% among colic patients and 1% in the “wounds” group, with an average rate of 2.6% (9/340) for both indications.
Conclusion: These results demonstrated a massive entry of MDR pathogens in equine clinics via colonized equine patients, implying a constant challenge to any hygiene management system.

The RESET-research database: A comprehensive collection of microbiological and epidemiological information on ESBL-producing bacterial strains from Germany

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Keywords: information depth, isolate characteristics, epidemiological analyses

Background and objectives: In the course of the research network RESET (www.resset-verbund.de), animals, humans, food and the environment were sampled to investigate the dissemination and characteristics of resistant *Enterobacteriaceae*. The depth of information of certain groups of isolates differs depending on the applied diagnostic methods. To be able to use the results of the isolate characterisations for overall epidemiological analyses the information depth of isolates needs to be analysed.
Materials and methods: Epidemiological data on origin of the samples and the results of the laboratory analyses of nine project partners were collected in a common hierarchical structured database. In addition to the basic characterisation the isolates were further characterised by in depth diagnostic methods. Using descriptive statistical methods, an overview of the existing data base information will be generated and displayed graphically.
Results: In epidemiological studies and from other strain collections 9,853 samples were collected in the period from 2008 to 2011. From these samples 3,095 bacterial strains have been isolated so far. In this contribution, the structure of the RESET research database and the different information depth of isolates will be presented.
Conclusion: Joint analyses of data collected in a common data base require a detailed knowledge of the data quality. This knowledge allows the targeted application of statistical methods such as multiphase sampling procedures.

Retrospective analysis addressing the emergence of the plasmid encoded colistin resistance gene *mcr-1* in German livestock farms during the years 2011-2013

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Keywords: multidrug-resistance, chicken, pig

In November 2015 the emergence of the first plasmid-encoded colistin resistance gene *mcr-1* was detected in animals as well as human beings in China. Within the last couple of months, a multitude of further studies was performed, indicating a global spread of the plasmid encoded resistance gene. As in human medicine, colistin is one of the last therapeutic options for the treatment of multidrug-resistant bacteria, the current situation has to be assessed critically. On the other hand in veterinary medicine, colistin is widely used for the treatment of diarrhoea in food-animals like pigs or poultry. Thus, indicating that the worldwide spread of the plasmid-encoded colistin resistance gene *mcr-1* reflects a major topic at the interface of human and animal health. To evaluate the *mcr-1* occurrence on farm level, bacterial cultures sampled on pig- as well as chicken-fattening farms during the years 2011 to 2013 were systematically screened for the presence of this gene. Primarily cultures deriving from pooled feces, boot swabs as well as dust samples, collected in a cross-sectional study including 58 pig-fattening and 45 chicken-fattening farms throughout Germany were investigated by PCR. Subsequently single *mcr-1* positive *E. coli* and one *K. pneumoniae* were isolated using MacConkey agar plates containing 2 µg/ml colistin. The received isolates are currently further investigated by using different phenotypic- as well as genotypic approaches. Based on the so far obtained results the *mcr-1* gene occurrence for the years 2011 to 2013 can be assessed at a value of 26% of the investigated pig- as well as 24% of the investigated chicken-farms. Ongoing and thorough investigations of the current situation in livestock farms are highly recommended.

Prevalence of extended spectrum and AmpC ß-lactamase producing *Enterobacteriaceae* in poultry during slaughter

P. von Tippelskirch[1], G. Gölz[1], S. Orquera[1], M. Projahn[2], K. Dähre[2], A. Friese[2], U. Rösler[2], T. Alter[1]

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Keywords: ESBL, AmpC, poultry

Background and objectives: Extended spectrum and AmpC ß-lactamase forming *Enterobacteriaceae* hydrolyse ß-lactam antibiotics, such as penicillines and cephalosporines. As food producing animals are considered a reservoir for these bacteria, meat represents a possible source of infection for humans. Other studies already showed high prevalence of ESBL/AmpC forming *Enterobacteriaceae* in poultry, however not much is known about the exact number of ESBL/AmpC-forming *Enterobacteriaceae* during slaughter.
Materials and methods: Samples of four chicken flocks were screened qualitatively and quantitatively for ESBL/AmpC producing *Enterobacteriaceae* using MacConkey agar containing cefotaxime. Species were identified by MALDI-TOF and antimicrobial susceptibility tested by disk diffusion assays. The presence of ESBL/AmpC genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$, *bla*$_{CMY}$) was determined by RT-PCR in all cefotaxime resistant *Enterobacteriaceae* isolates.
Results: On average, 40 % (40/100) of caecal, 75 % (75/100) of skin, 23 % (23/100) of meat and 35 % (18/52) of environmental samples contained ESBL/AmpC forming *Enterobacteriaceae*. The median number of the cefotaxime resistant *Enterobacteriaceae* was higher in caecum and skin (1×10^3 CFU/g) than in meat (7×10^1 CFU/g).
Conclusion: Although prevalence was high during poultry slaughter, total number of ESBL/AmpC forming *Enterobacteriaceae* was lower than expected.

Does size matter? - Transferability of extended-spectrum β-lactamase gene-carrying plasmids harboured by *Escherichia coli* isolates of diseased food-producing animals

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Keywords: β-lactams, horizontal gene transfer, co-selection of antimicrobial resistance genes

Background and objectives: Extended-spectrum β-lactamase (ESBL) genes are commonly disseminated by plasmids. This study aimed at the characterization of ESBL gene-carrying plasmids of *Escherichia coli* isolates from diseased food-producing animals.
Materials and methods: ESBL genes were identified by PCR/sequencing in 324/2,896 bovine, 75/1,562 porcine and 20/2,391 avian isolates, all originating from diseased animals of the GE*RM*-Vet program. Representative isolates (n=90) and their plasmids were characterized by susceptibility testing, XbaI-PFGE, multilocus sequencing typing (MLST), phylotyping, transfer experiments, replicon typing, S1-nuclease and PCRs for the detection of resistance genes.
Results: The 90 ESBL-producing *E. coli* displayed mostly unrelated XbaI-patterns (n=78) and unique MLST sequence types (n=27). Their ESBL gene-carrying plasmids belonged mainly to the incompatibility groups IncF (n=27), IncI1 (n=22) or IncN (n=19), and 86/90 plasmids were conjugative. IncN plasmids carried $bla_{CTX\text{-}M\text{-}1}$ genes, showed the smallest sizes (35-50 kb), the highest conjugation efficiencies (18/19, $1.3x10^{-2}$-$7.8x10^{-3}$) and only two plasmids carried co-located resistance genes, *aac(3)-IVa* or *qnrS1*. In contrast, IncF-FIA-FIB plasmids carried $bla_{CTX\text{-}M\text{-}15}$ genes, were large (165-180 kb), displayed low conjugation efficiencies ($3.1x10^{-5}$-$9.0x10^{-7}$) and showed multi-resistance.
Conclusion: This study highlights the importance of especially IncN plasmids in the dissemination of ESBL genes.

ESBL-/AmpC-producing Enterobacteriaceae in the broiler production chain

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Keywords: ESBL, Antibiotic resistance, Broiler

Background and objectives: Previous studies showed that *Enterobacteriaceae* producing extended-spectrum (ESBL) or AmpC beta-lactamases are occurring in herds on broiler farms. Even one day old chicks were tested positive for these resistant bacteria suggesting an early entry within the broiler production chain. In our study we, therefore, surveyed seven parent broiler flocks and their chicks along the broiler fattening period.
Materials and methods: First we investigated faecal and environmental samples from the parent flocks. Several samples from the environment of the hatchery as well as from the eggs where taken. At least the hatched chicks were investigated three times during the fattening period. We isolated suspicious enterobacteria from the samples and analysed their species and the ESBL-/AmpC genes.
Results: From six out of seven flocks we isolated ESBL-/AmpC producing Enterobacteria from the chicks during the fattening period which in nearly all cases differ genetically from those we found in their parent flocks. However, we could provide evidence for a pseudo-vertical transfer from a parent flock to the hatchery via contaminated egg surfaces.
Conclusion: An exclusively vertical transfer of ESBL-/AmpC producingenterobacteria along the broiler production chain seems to be not very likely. The management of the hatchery as well as the cleaning and disinfection procedures on the fattening farm also have an impact on the occurrence of ESBL-/AmpC producing Enterobacteria.

Session 5: Innate and Adaptive Immune Response

October 14, 2016
09:00 – 10:30

Room Steglitz
Chairs: Veronika von Messling and Stephan Ludwig

Fruit bat tetherin restricts release of virus-like particles and is inefficiently antagonized by filovirus glycoproteins

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Keywords: Tetherin, Filovirus, Fruit bat

Tetherin is an interferon-induced cellular protein that can inhibit budding of enveloped viruses from infected cells. Many viruses developed strategies to counteract tetherin and filoviruses employ their glycoproteins (GPs) to antagonize tetherin in human cells. Tetherin homologues are found in a broad variety of species ranging from primates to bats and fruit bats have been identified as a natural reservoir of filoviruses. Here, we analysed whether filovirus GPs differ in their abilities to counteract tetherin of human and bat origin.
We studied inhibition of virus-like particle (VLP) release by tetherin and its counteraction by filovirus GPs employing a previously published VLP system.
We found that tetherin homologues amplified from two bat species, *Epomops* and *Hypsignathus*, inhibited VLP release from transfected cells with higher efficiency than human tetherin. Interestingly, the HIV-1 accessory protein Vpu and filovirus GPs antagonized release restricting activity of human tetherin, in keeping with published data, while counteraction of bat tetherins was inefficient or absent. Moreover, analysis of chimeric proteins suggested that antagonism of human tetherin by GP depends on intra- and extracellular sequences while antagonism by Vpu depends exclusively on intracellular sequences. Collectively, the antiviral activity of fruit bat tetherin is inefficiently antagonized by filovirus GPs, suggesting tetherin may contribute to control of viral spread in the reservoir host.

An inactivated bivalent rabies - canine distemper virus vaccine induces protective immunity against both viruses in ferrets

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Keywords: rabies virus, canine distemper virus, bivalent vaccine

Background and objectives: Rabies (RABV) and canine distemper viruses (CDV) can cause lethal disease in domestic and wild carnivores. While safe inactivated rabies vaccines are available, the live-attenuated distemper vaccines retain residual virulence in highly susceptible species. The objective of this study was to assess the efficacy of an inactivated RABV vaccine that concurrently displays the CDV glycoproteins in ferrets.

Materials and methods: Beta-propiolactone-inactivated recombinant RABV particles expressing CDV glycoproteins from different strains were generated and characterized *in vitro*. To evaluate the immunogenicity against RABV and CDV, we immunized ferrets with a single dose or a prime/boost regimen of the respective vaccine candidate and followed the antibody responses against both viruses. All animals were then challenged with a wild-type CDV strain.

Results: All immunized animals developed protective levels of RABV neutralizing antibodies within three weeks after the first immunization, while anti-CDV antibodies in the protective range were only detected after the boost. Animals vaccinated with vectors containing both CDV glycoproteins survived the challenge. In contrast, immunization with a RABV displaying the more variable CDV vaccine attachment protein alone did not protect from infection with a more recent wild type strain.

Conclusion: An inactivated bivalent rabies-based CDV vaccine can induce protective immune responses against both pathogens.

Bat Mx proteins: evolution and antiviral specificity

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Keywords: innate immunity, bat Mx proteins, antiviral activity

Bats are a reservoir for various, often zoonotic viruses. These viruses rarely cause clinical symptoms in bats. The interferon system plays a key role in controlling viral replication. It induces the expression of antiviral myxovirus resistance (Mx) proteins. We hypothesize that Mx proteins act as important factors controlling viral replication in bats. To this end, we characterized bat Mx proteins from three selected bat families for phylogenetic and functional analysis.
Bat cell lines were analysed for their Mx expression. The ORFs of the Mx cDNAs were amplified, cloned and investigated for evolutionary relationships, allelic variations and positive selection. Bat Mx proteins were tested for their antiviral activity.
Mx was expressed in bat cell lines upon stimulation with interferon. Bat Mx1 proteins inhibited the polymerase activity of FLUAV, VSV and EBOV. Phylogenetically, the bat Mx1 and Mx2 sequences clustered closely with the human orthologs MxA and MxB. We were able to identify allelic variations within the Mx genes for all analyzed bat species. Positive selection occurred in distinct regions of the bat Mx1 and Mx2 genes indicating species-specific adaptation processes.
Bat Mx genes are quite similar to human MxA and MxB. Their gene products are antivirally active against a variety of viruses. They show patterns of positive selection suggesting a coevolution with viral pathogens and a role in the control of virus replication in bats.

The immunopathogenic potential of *Arcobacter butzleri* – pathogen or commensal?

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Keywords: Arcobacter butzleri, immunopathogenic potential, pathogen-host interactions

Background and objectives: Given that only limited information is available about the immunopathogenic properties of *Arcobacter* infection, we here compared *A. butzleri* with intestinal pathogenic and commensal species *in vivo*.
Materials and methods: Gnotobiotic IL10-/- mice were generated by broadspectrum antibiotics and perorally infected with *A. butzleri, C. jejuni* or a commensal intestinal *E. coli* strain.
Results: Either strain stably colonized the murine intestines upon infection. At day 6 postinfection, only *C. jejuni* infected mice displayed clinical sequelae such as wasting bloody diarrhea. Gross disease was accompanied by increased numbers of colonic apoptotic cells and distinct immune cell populations. Whereas *A. butzleri* and *E. coli* infected mice were clinically unaffected, colonic immune cell numbers increased in the former, but not in the latter. Both *A. butzleri* and *C. jejuni* induced increased secretion of proinflammatory cytokines in large and small intestines. Remarkably, even though viable bacteria did not translocate from the intestines, systemic immune responses were induced in *C. jejuni*, but also *A. butzleri* infected mice as indicated by increased pro-inflammatory cytokine concentrations in serum samples.
Conclusion: *A. butzleri* induce less distinct proinflammatory sequelae as compared to *C. jejuni*, but more pronounced local and systemic immune responses than commensal *E. coli* indicating that *A. butzleri* is more than a commensal in vertebrate hosts.

Correlating viral interferon antagonism with host evolutionary distance by a trans-species comparative interferon bioassay

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Keywords: Interferon, host range, antagonistic efficiency

Viral interferon (IFN) antagonism influences the host range of viruses. The quantitative proportions of viral countermeasures against IFN responses in different hosts (e.g., typical reservoir hosts vs. accidental hosts) might correlate with inter-host evolutionary distances.

We established an IFN bioassay-based protocol allowing a trans-species quantitative comparison of cellular innate antiviral immunity. Two highly active IFN antagonists from viruses with a broad host tropism, Influenza A virus NS1 and Rabies virus phosphoprotein, were directly compared by IFN bioassay in 9 cell lines representing 9 species of 6 vertebrate orders. The viral antagonists were provided by lentiviral transduction and cells were subsequently stimulated by dsRNA analogues. In parallel, GFP-lentivirus-transduced cells were stimulated and used as reference to determine the maximal IFN level and to allow the species-wide comparison of relative IFN levels (rIFN-l).

The detected rIFN-l ranged from 0.5 to 3, whereby low rIFN-l represented high anti-IFN efficiency. Highest anti-IFN efficiency (rIFN-l<1) was found in cell cultures of designated reservoir species ($p \leq 0.01$ for both IFN antagonists). The anti-IFN efficiency correlated inversely with phylogenetic topology, represented by pairwise patristic distances between the respective hosts.

Our results suggest that patristic distance between reservoir host and target host may forecast the efficiency of viral interferon antagonism in the target host.

Exploitation of PKR as a selector for the identification of immunostimulating influenza virus RNAs useful for antiviral therapy

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Keywords: PKR, influenza virus, viral RNA

Background and objectives: Current antivirals against influenza A viruses (IAV) are limited to few viral target structures and suffer from rapid development of resistant virus mutants. Alternative treatment strategies could utilize the capability of the cellular defense system to recognize and counteract an infection. Thus, sensors like RIG-I or the RNA-activated protein kinase R (PKR) detect conserved viral RNA structures and mount an efficient immune response.
Here, we employ PKR to identify novel lead structures for the development of antiviral therapeutics.
Material and Methods: PKR was immunoprecipitated from IAV-infected cells and the associated RNA was isolated. Individual RNAs were cloned and amplified by *in vitro* transcription.
Results: PKR-bound RNA from IAV-infected cells stimulated both PKR and RIG-I, indicating an overlap in target requirements for both sentinel pathways. Furthermore, transfection of PKR-derived RNA conferred a broad antiviral protective effect to target cells, reducing the replication of oseltamivir-resistant IAV or VSV by several logs. Individual cloned RNAs were examined to identify the most potent stimulators and to delineate responsible motives. Only RNAs consisting of entire IAV segments were strong stimulators of the antiviral response, while engineered RNAs lacking one segment end were not.
Conclusion: PKR is a useful selector for the identification of immunostimulating viral RNAs with potential for the development of novel therapeutics.

Session 6: Novel methods, diagnostics and NGS

October 14, 2016
09:00 – 10:30

Room Zehlendorf
Chairs: Katrin Kuhls and Martin H. Groschup

Automated whole genome sequence based serogrouping of *Listeria monocytogenes* isolates

P. Hyden[1,3], A. Pietzka[1], A. Lennkh[1], A. Murer[1], B. Springer[1], A. Indra[1], D. Harmsen[2], F. Allerberger[1], C. W. Sensen[3], W. Ruppitsch[1]

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Keywords: Listeria monocytogenes, cgMLST, serogrouping

Background and objectives: Whole genome sequencing (WGS) is becoming the primary tool for identification, characterisation and comparison of bacterial isolates. The aim of this study was to extract the respective serotype information from WGS data, because serotyping is still a first level subtyping tool in public health laboratories.

Materials and methods: A core genome (cg)MLST scheme consisting of 1,701 target genes (*JCM* 2015, 53: 2869) was applied for NGS data interpretation of 217 *L. monocytogenes* isolates comprising all 12 serotypes using SeqSphere$^+$ (Ridom, Germany).

For serogroup determination task templates were created in SeqSphere$^+$ using gene targets as described by Doumith *et al.* (2004) and targets identified via a Python script that extracted serogroup information from allelic profiles.

Results: 204 isolates (94%) could be correctly assigned to serogroups using the serogroup specific task templates. A minimum spanning tree was calculated based on cgMLST in SeqSphere$^+$. Isolates clustered according to their serotypes and serogroup specific cluster types (CTs) were identified. In addition to the five published serogroup-specific genes studying all loci in the cgMLST and the accessory genome identified another 35 specific targets.

Conclusion:

In this study we successfully used WGS data for *in silicio* assignment of isolates to serogroups as first level information for public health laboratories representing an additional benefit of WGS technology.

Next-Generation Multiplex PCRs for Virus Diagnostics

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Keywords: Targeted enrichment for next-generation sequencing, virus diagnostics, AmpliSeq multiplex PCRs

Next-generation sequencing (NGS) technologies offer great chances for the diagnosis of viral infections in humans. NGS allows parallel screening of numerous patient samples and requires no a priori knowledge of the pathogen. However, the sensitivity of viral sequence identification is limited, given that the viral load of patient samples can be low and the background of human genetic material is high. Recent attempts to increase the sensitivity of virus detection with NGS have led to the development of targeted sequence enrichment techniques, including sequence capture and pre-amplification.

In the present study, we have assessed the feasibility of multiplexed sequence amplification prior to NGS to facilitate the identification of viral pathogens in complex patient samples. We have developed a combination of ultrahigh-multiplex PCRs and NGS, which is able to target more than 500 viral genomic regions in a single approach.

Using multiplexed pre-amplification prior to NGS, we were able to diagnose viruses present in as little as one genomic copy in a sample of human genetic material. Furthermore, we combined ultrahigh-multiplex PCRs with real-time nanopore sequencing, generating sufficient viral read numbers for species identification within sample-to-result times of less than 5 hours.

We conclude that ultrahigh-multiplex PCRs prior to NGS increase the sensitivity of virus detection in patient samples, decrease the amount of nucleic acid input and reduce times of sample preparation, sequencing and analysis.

Tracing the geographical origin of human brucellosis in Germany

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Keywords: brucellosis, whole genome sequencing, trace-back analysis

Background and objectives: Brucellosis is one of the most common bacterial zoonotic diseases globally, although Germany is considered a brucellosis-free area with less than 50 cases reported annually. Increasing case numbers in 2014 and 2015 raised the question to the geographical origin of strains isolated in Germany.
Materials and methods: Culture-positive cases of human brucellosis confirmed by the national Brucella consultant laboratory from 2014 henceforward were selected for whole genome sequencing (WGS) using Nextera XT Library kit and MiSeq platform. After de-novo and reference-based assembly, genome comparison with genome-wide SNP analysis was applied and genetic determinants of antibiotic resistance were investigated. Moreover, in silico multilocus VNTR analysis (MLVA) was performed.
Results: Phylogenetic analyses using whole-genome SNPs revealed spatial clustering. MLVA congruently grouped the isolates and predominantly matched the East Mediterranean genetic clade. Genetically encoded antibiotic resistance determinants with phenotypic effect on the susceptibility of commonly used substances for brucellosis treatment could not be observed.
Conclusion: This study comprises the most comprehensive collection of B. melitensis strains isolated from humans in Germany during the last three years and illustrates the essential role of WGS-derived bacterial typing in cluster investigation. However, epidemiological information and reliable metadata in used databases are crucial.

Revolutionizing outbreak investigations: a retrospective analysis of nosocomial LA-MRSA transmission in an Austrian hospital by next generation sequencing

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Keywords: LA-MRSA, next generation sequencing, subtyping

Background: From 2004 onwards in Austria a steady increase of human CC398 LA-MRSA isolates (2004: 0.2%; 2015: 7.9%) was observed. In December 2010 a cluster of seven cases of colonization/infection with LA-MRSA *spa*-type t011, ST398 was identified among hospital patients. Epidemiological findings indicated MRSA acquisition through physical contact to pigs for cases I-III, and through contact (environment, health care workers) to cases I-III for cases IV-VII. Aim of our study was to reanalyze this cluster by using next generation sequencing (NGS).
Material/methods: DNA libraries were made from 31 LA-MRSA isolates by using a NexteraXT kit. Fragments were sequenced on a MiSeq (Illumina Inc). A core genome MLST scheme comprising 1,862 target genes was applied for interpreting the NGS results using SeqSphere+ (Ridom, Germany).
Results: NGS based typing clearly excluded case II from the outbreak, falsified the transmission of the outbreak strain from case V to VI and identified the transmission from case III to V, and from case VI to case VII.
Conclusions: Core genome MLST allows subtyping of LA-MRSA *spa*-type t011 isolates and is of benefit in investigating epidemiological clusters. NGS allows an extended analysis of chains of transmission with the highest possible resolution and will noticeably enrich findings of epidemiological investigations and former molecular typing of MRSA. As a consequence, NGS should be used as a standard typing method to enhance infection control.

Crimean Congo Haemorrhagic Fever, 2014 Sudan

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**Authors contributed equally*

In 2014, an outbreak of hemorrhagic fever in humans was reported from different states of Sudan (South Darfur, West Kordofan, South Kordofan). The NPHL, Khartoum investigated the cases and forwarded 28 sera samples from patients suffering from hemorrhagic fever to the RKI. The sample-set included a panel of 10 sera collected during former hemorrhagic fever outbreaks in the same region in 2013. All sera were tested with qPCR assays for Marburg virus, Ebola virus and CCHFV. Additionally, all samples were subjected to metagenomic deep sequencing on an Illumina MiSeq sequencer.

CCHF was identified by two independent qPCR assays in a sample from November 2013 and November 2014, respectively. Deep sequencing confirmed these results. Based on the available sequences the novel CCHFV strain 'Sudan 2014' shares 96% identity (na) with its closest relative CCHFV SPU 187/90 from South Africa.

CCHFV is reported to be transmitted by ticks in Europe, Asia and Africa and known as etiological agent of severe hemorrhagic fever in humans and livestock. Beside insect repellent no preventive measures are available. The pathogenicity and characteristics of this novel strain have yet to be determined by cell culture isolation and serology. Further molecular analysis will contribute to clarify the divergence of the CCHFV strains detected in 2013 and 2014. First results including novel viruses identified by metagenomics will be presented.

Microfluidically supported organoids for modelling systemic inflammation and infection

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Keywords: inflammation, organ-on-a-chip, in vitro model

Background and objectives: Animal models are frequently used to study effects of zoonotic agents. However, recently a controversial debate about the transferability of data obtained in mouse models to human conditions emerged. Although cell-based *in vitro* approaches can be an alternative, conventional cell culture methods hardly reflect cellular cross-communication and neglect essential physiological parameters of the living organism.
Materials and methods: We developed biochip-embedded organoid models of the liver, gut and brain. Dynamic perfusion of biochip-embedded tissues allows an optimal supply with nutrients and oxygen, an efficient removal of catabolic metabolites, and enables a physiological cell polarization and communication within tissues.
Results: The modular approach of the biochip design permits a variable microfluidical interconnection of different organoid models in a freely selectable arrangement. The organoids are composed of essential human cell types of the respective organs and were tested in the context of inflammation. We demonstrated that the three-dimensional tissue models are able to resemble cellular and molecular events of infection-related organ-dysfunction as well as processes of immune cell-related tissue repair.
Conclusion: The biochip-based organoid models are thus valuable tools for studies of systemic infection with zoonotic agents and help to improve the predictability and transferability of in vitro studies to human conditions.

Session 7: New and re-emerging zoonoses

October 14, 2016
11:00 – 12:30

Room Ballsaal
Chairs: Christian Drosten and Rainer Ulrich

Poultry-associated multi-drug resistant *Salmonella* spp., *Campylobacter* spp. and *Arcobacter* spp. in urban Ghana

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Keywords: Poultry, MDR, enteric Bacteria

Frequent overuse of antibiotics in animal farming has contributed to the emergence of antibiotic resistance. Meat contaminated with *Salmonella*, *Campylobacter* and *Arcobacter* may infect humans and lead to emergence of MDR. Although the transmission mode for the named bacteria is known (faecal-oral), it remains speculative which pathogen vehicles are predominant in Africa. This study investigated contamination of local and imported chicken with MDR *Salmonella*, *Campylobacter* and *Arcobacter* spp.. Frozen and fresh meat was cultured using standard media. Bacteria were identified by microscopy and biochemistry. Antibiotic susceptibility (AST) was tested according to CLSI guidelines. In total, 200 samples were collected. The highest contamination was seen for *Arcobacter* (n=50; 25%), followed by *Salmonella* and *Campylobacter* (each n=17, 8.5%). Contamination for all bacteria was highest for local meat: *Salmonella*, *Campylobacter* and *Arcobacter* were isolated from 76.5% (n=13), 86% (n=43) and 82.4% (n=14) of local samples, respectively. AST revealed that 23.5% (n=4) of *Salmonella* were MDR. Resistance to Fluorquinolones (FQ) for *Salmonella*, *Campylobacter* and *Arcobacter* was identified in 62.5%, 76.5% and 52% of isolates respectively. This study highlights that despite concerns of contamination risks due to imports of meat, contamination was highest for local meat. Of major concern is the high level of FQ resistance seen as this may lead to emergence of FQ resistance in the human population.

Link of a ubiquitous human coronavirus to dromedary camels

I. Eckerle[1], V.M. Corman[1,2], Z. Memish[3], A.M. Liljander[4], R. Dijkman[5,6], H.R. Jonsdottiert[5,6], K. Juma Ngeiywah[7], E. Kamau[7], M. Younan[8], M. Masri[9], A. Assiri[9], I. Gluecks[10], B.E. Musa[11], B. Meyer[1], M.A. Müller[1], M. Hilali[12], S. Bornstein[13], U. Wernery[14], V. Thiel[5,6], J. Jores, J.F. Drexler[1,2], C. Drosten[1,2]

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Keywords: Coronavirus 229E, dromedary camel, zoonotic transmission

Background and objectives: While the Middle East respiratory syndrome Coronavirus (MERS-CoV) is an emerging CoV with zoonotic origin, little is known about the origin of the four endemic CoVs.
Materials and methods: Respiratory swabs from dromedaries were screened by targeted RT-PCR. Sera were screened for antibodies by indirect immunofluorescence assay. Virus isolation was performed on PCR-positive samples.
Results: We found viruses related to HCoV-229E in 5.6% of 1,033 animals. Human and dromedary-derived viruses are monophyletic, suggesting ecological isolation. One gene of dromedary viruses existed in two versions in camels, full length and deleted, whereas only the deleted version exists in humans.
Live isolates of dromedary viruses were obtained. The viruses utilized the human receptor Aminopeptidase N. Inefficient replication in several mucosa-derived cell lines and airway epithelial cultures suggested lack of adaptation to the human host. Dromedary viruses were sensitive to the human type I interferon response. Antibodies in human sera neutralized dromedary-derived viruses, suggesting population immunity.

Conclusion: While no epidemic risk seems to emanate from these viruses, evolutionary inference suggests that the endemic human virus HCoV-229E may constitute a descendant of camelid-associated viruses. This scenario provides a reminder of a potential trajectory of emergence for the MERS-CoV.

Culex pipiens and *Culex torrentium* mosquitoes from Germany are susceptible to infection with West Nile virus

M. Leggewie[1], M. Badusche[1], M. Rudolf[1], S. Jansen[1], J. Börstler[2], R. Krumkamp[3,4], K. Huber[1,5], A. Krüger[6], J. Schmidt-Chanasit[2,4], E. Tannich[4,7], S. C. Becker[1,8]

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Keywords: WNV, Culex, Germany

West Nile Virus (WNV), a flavivirus with an avian primary host, poses a serious public health risk to Europe. *Culex* mosquitoes include many potential vectors for WNV, of which some are also native to Germany. However, their potential role in establishing an enzootic WNV transmission cycle is so far unknown.

Accordingly, we have analysed susceptibility of German *Culex* mosquitoes for WNV, with particular interest on field-collected *Culex torrentium and Culex pipiens* biotype *pipiens*.

Egg rafts of *Culex* mosquitoes from two geographically distinct sampling areas in Germany were collected and differentiated by molecular methods. Adult females, reared from these egg rafts, were challenged with WNV via artificial blood meals. WNV infection was confirmed by real-time RT-PCR and virus titration.

The results indicate that field-collected *Culex pipiens* biotype *pipiens* as well as *Culex torrentium* native to Germany are susceptible to WNV infection at 25 °C or 18 °C incubation temperature. *Culex torrentium* mosquitoes were the most permissive species with maximum infection rates of 96 % at 25 °C. Disseminating infections were at 25 °C were found in up to 94 % of *Culex pipiens* biotype *pipiens* and in up to 100 % of *Culex torrentium*. Notably, *Culex pipiens* biotype *pipiens* from Southern Germany were more susceptible to WNV infection than those from Northern Germany.

All in all, we observed high infection and dissemination rates even at 18 °C, qualifying German *Culex* mosquitoes as potential vectors for WNV.

Detection of *Mycobacterium tuberculosis* complex bacteria in feces, urine and saliva of red deer *(Cervus elaphus)* and cattle from the hotspot regions in Germany

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Keywords: bovine Tuberculosis, red deer, transmission

The recently increasing number of bovine tuberculosis especially in the Allgäu region raised questions regarding transmission routes and shedding pathways. Earlier investigations revealed that Mycobacteria of the tuberculosis complex (MTC) could be found only in single lymph nodes in about 95 % of the positive tested red deer; generalized or severe lung tuberculosis (in human medicine the only form considered to pose a transmission risk) was very rarely diagnosed. Therefore, a transmission solely by aerosols cannot explain a high number of infected animals without reports about coughing. The aim of our project was to find out whether MTC can also be shed by secretions and excretions even if no active tuberculosis is present.

For this purpose, non-standardized feces/urine/saliva samples of red deer from hotspot regions in Germany were analyzed for MTC after necropsy. A total of 980 pathologically negative and 21 positive animals were examined by real time PCR; positive samples also by cultivation. Additionally, cattle from a farm in the hotspot region showing positive, negative and suspicious tuberculin skin tests were sampled. Feces/blood/milk/saliva from cattle (n=13) and feces/blood/saliva from goats (n=3) were investigated by real time PCR and culture.

MTC bacteria (*M. caprae*) were detected in feces of one red deer, but in none of the samples of cattle and goats.

This is the first report on the detection of MTC in feces of a naturally infected red deer in Europe. However, transmission mechanisms remain still unclear and need further investigation.

Discovery of novel bunyaviruses in mosquitoes from the neotropics

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Keywords: bunyavirus, arbovirus, insect-specific virus

Background and objectives: Orthobunyaviruses are mainly mosquito-borne and infect a wide range of vertebrates. The proposed new genus *Herbevirus* contains insect-specific viruses defining a phylogenetic sister group to orthobunyaviruses.
Materials and methods: In total 3491 mosquitoes were tested for orthobunyavirus-like infection by a generic RT-PCR. Full genomes were obtained by deep-sequencing. Virus isolation was done in insect cells. Virus growth was analysed in insect, vertebrate and avian cells.
Results: Three bunyaviruses were identified in a single mosquito each. We found Wyeomyia virus (WYOV), tentatively named WYOV strain Palenque, a novel orthobunyavirus named Baakal virus (BKAV) and a novel herbevirus named Lakamha virus (LAKV). Genome based phylogenetic analyses placed LAKV on a long branch in basal relationship to herbeviruses and BKAV grouped between the Koongol and Turlock serogroups. BKAV was isolated in mosquito cells. Replication in cells derived from vertebrates was only found in duck cells suggesting a potential mosquito-bird-transmisson cycle. Replication of the prototype mosquito-borne virus La Crosse virus and of BKAV was not affected by higher temperatures whereas replication of the insect-specific Herbert virus was blocked at temperatures over 31°C.
Conclusion: Growth curve analyses in insect cells can provide a surrogate modell to differentiate between mosquito-borne and mosquito-specific novel viruses.

In vivo screening for the novel zoonotic bornavirus (VSBV-1): New positive squirrel species and further sequences

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Keywords: variegated squirrel bornavirus 1, encephalitis, squirrel

The recently discovered novel zoonotic bornavirus, tentatively designated as variegated squirrel bornavirus 1 (VSBV-1), caused fatal encephalitides. Viral RNA was detected in brain samples from the patients as well as in samples of a contact squirrel (*Sciurus variegatoides*). The aim of our ongoing investigations is to identify the reservoir(s) of VSBV-1 and to understand its transmission to humans. For this purpose, a non-invasive, *in vivo* sampling method for squirrels and a VSBV-1 specific real-time RT-PCR were established.

Oral swab and fecal samples of different squirrel species were screened using VSBV-1 specific RT-qPCR. Positive screening results were confirmed by testing the corresponding brain samples collected after euthanasia. Whole genomes were determined by classical and next-generation sequencing.

In 3.5% of the tested samples, VSBV-1 RNA was detected. Samples from both variegated squirrels (*Sciurus variegatoides)* and beautiful squirrels (*Callosciurus)* were positive. The results corresponded between the *in vivo* sampling (fecal/oral) and the *post mortem* organ samples. The whole genomes generated clustered within the putative new bornavirus species *Mammalian 2 bornavirus*.

In conclusion, VSBV-1-diagnostics allows the reliable and rapid screening of live animals. VSBV-1 RNA could be detected in multiple squirrels of different species forming a genetic cluster. Further studies will have to evaluate e.g. the host origin and specificity of this novel zoonotic bornavirus.

Session 8: Parasites

October 14, 2016
11:00 – 12:30

Room Steglitz
Chairs: Anton Aebischer and Karsten Nöckler

The host cell carbohydrate metabolism regulates *Toxoplasma gondii* bradyzoite formation in skeletal muscle cells

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Keywords: Toxoplasma gondii, stage conversion, metabolism

Chronic infections in intermediate hosts are critical for the zoonotic life cycle of *Toxoplasma gondii* and for pathogenesis of human toxoplasmosis. It requires parasite differentiation to the latent bradyzoite stage and cyst formation in neuronal and muscle tissue. The aim of this study is to unravel host cell prerequisites for *T. gondii* stage differentiation in skeletal muscle cells (SkMCs).

RNAseq and GC-MS were used to characterize expression and activity of carbohydrate metabolic enzymes in terminally differentiated myotubes and proliferating myoblasts, which do or do not sustain *T. gondii* bradyzoite formation, respectively. The impact of distinct metabolic traits of the host cells were functionally analysed using pharmacological inhibitors.

T. gondii-infected and non-infected myoblasts presented higher mRNA levels of enzymes of the pentose phosphate pathway (PPP) whereas myotubes expressed higher levels of glycolytic enzymes and the pyruvate carboxylase. Metabolic flux analyses revealed higher PPP activity in infected and non-infected myoblasts whereas myotubes preferentially channelled glycolytic carbon into the TCA cycle via anaplerosis. Importantly, inhibition of the PPP enzymes G6PDH and 6P-gluconate dehydrogenase accelerated *T. gondii* bradyzoite formation particularly in myoblasts.

These data uncover the host cell metabolism as regulator of *T. gondii* stage differentiation and might explain tissue predilection of parasite tissue cysts in intermediate hosts.

Epidemiology and genetic characterization of Giardia spp. infections in wild rodents in Germany

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Keywords: Giardia spp, wild rodents

Background and objectives: Protozoan parasites of the genus *Giardia* are ubiquitously found in humans and animals. *Giardia duodenalis* is considered as a species complex that consists of eight distinct phylogenetic sub-groups (assemblages A-H). Assemblages A and B infect various mammals, including humans and rodents and are potentially zoonotic. Rodents can additionally harbour *G. duodenalis* assemblage G, *G. muris* and *G. microti*.
We aim at characterization of *Giardia* spp. in wild rodents in Germany to determine potentially zoonotic *G. duodenalis* assemblages in these animals.
Materials and methods: 581 fecal samples from wild rodents were sampled in distinct regions of Germany and analysed for the presence of *Giardia* by immunofluorescence microscopy, qPCR, and semi-nested PCR and sequence analysis at the small subunit rDNA locus (SSU rDNA).
Results: Most animals belonged to one of the three genera *Apodemus* (16%), *Microtus* (31%) or *Myodes* (52%). A significantly lower *Giardia* prevalence in *Apodemus* (26%-43%) compared to *Myodes* (63%-91%) and *Microtus* (80%-94%) was detected. *Microtus* and *Myodes* were predominantly infected with *G. microti*, but *Apodemus* with *G. muris*. *G. duodenalis* assemblage A was only detected in three animals, assemblage B in one animal.
Conclusion: *Giardia* spp are highly prevalent in wild rodents in Germany. *G. microti* or *G. muris* predominate in different rodent genera. Infections with potentially zoonotic *G. duodenalis* assemblages appear very rarely.

Relationship between the seroprevalence in chicken and the presence or infectivity of *Toxoplasma gondii* cysts in chicken meat and other edible tissues

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Keywords: Toxoplasma gondii, chicken, risk factors

Background and objectives: Meat appears to be a major source of *Toxoplasma gondii* infections in Europe. To gain more insight into the role of meat as a source of human infection with *T. gondii*, it is important to have an indication on the prevalence of infectious tissue cysts in the main livestock species. Serological assays are commonly used to determine the prevalence but the predictive value of seropositivity with respect to the presence of infective tissue cysts in various livestock species is largely unknown. The present study aimed at the identification of seropositive and seronegative chickens from organic and backyard farming.

Materials and methods: 32 laying hens, which had tested seropositive in an in-house TGSAG1-ELISA, were selected from more than 400 hens sampled on these farms. Hearts and limb muscles of seropositive chickens were homogenized, treated with an acid pepsin solution and examined for *T. gondii* tissue cysts in a bioassay with immunocompromised mice. In addition, 29 hens, which were seronegative in the TGSAG1-ELISA, were selected and examined in the same way.

Results: Viable *T. gondii*, all belonging to clonal lineage II, could be isolated from the majority of seropositive but from few seronegative chickens. Most *T. gondii* isolates were obtained in the bioassay using chicken heart samples, while limb muscles were rarely positive.

Conclusion: Serological assays can be used to identify chicken which harbour viable *T. gondii* in edible tissues.

Abundance of *Ixodes ricinus* and the prevalence of Lyme *Borrelia* pathogenic to humans

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Keywords: Borrelia burgdorferi sensu lato, Ixodes ricinus, Lyme disease

Lyme disease (LD) is a zoonotic, bacterial infection in humans caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex, transmitted by *Ixodes ricinus* ticks. This study focusses on LD prevention by examining the distributional patterns and the activity of questing *Ixodes ricinus*. From 2010-2012, the survey was performed at three peri-urban study sites in Berlin (Gatow, Tegel, Wannsee) at different spatial scales (nano, micro, macro). Additionally, the prevalence of genospecies pathogenic to humans was determined in questing ticks. This prevalence of infection in conjunction with tick densities results in a theoretical exposure risk for people. When comparing the tick activity between study sites for 2010-2012 at the micro-macro scale, Tegel appeared to be dominated by nymphal ticks and Gatow by adult ticks. The highest prevalence of *Borrelia* sp. in questing ticks was detected at the Wannsee study site, where it exceeded the European average. The results illustrate that the activity of questing ticks and their prevalence of pathogenic borreliae varies tremendously at a small spatial scale. Keeping this in mind, risk maps for borreliae-infected ticks seem to be of little use. Risk assessment for people should be based on the theoretical risk of exposure, determined locally. The methods developed in this study may be applied in further investigations when evaluating and comparing the heterogeneity of questing ticks and prevalence of the causative agent of LD within or between different study sites. These future investigations may provide a basis for targeted landscaping in the sense of the One Health initiative.

Host-feeding patterns of mosquito species in Germany

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Keywords: Culicidae, Host species, Host-feeding pattern

Background and objectives: Mosquito-borne pathogens are of growing importance in many countries of Europe including Germany. At the same time, the transmission cycles of most mosquito-borne pathogens are not completely understood. There is especially a lack of knowledge about the vector capacity of the different mosquito species, which is strongly influenced by their host-feeding patterns.
Materials and methods: From 2012 to 2015, 775 blood-fed mosquito specimens were collected and analysed for their hosts. The host species for each mosquito specimen was identified with polymerase chain reactions and subsequent Sanger sequencing of the cytochrome b gene.
Results: A total of 32 host species were identified for 23 mosquito species, covering 21 mammalian species (including humans) and eleven bird species. Despite the collected blood-fed mosquito species had a strong overlap of host species, two different host-feeding groups were identified with mosquito species feeding on (i) non-human mammals and humans or (ii) birds, non-human mammals, and humans, which make them potential vectors of zoonotic pathogens only between mammals or between mammals and birds, respectively.
Conclusion: The presented study indicates a much broader host range compared to the classifications found in the literature, which highlights the need for studies on the host-feeding patterns of mosquitoes to further assess their vector capacity and the ecology of zoonoses in Europe.

Comparison of different commercial DNA extraction kits to detect *Echinococcus multilocularis* eggs in faecal samples from foxes

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Keywords: Echinococcus multilocularis, PCR, DNA extraction kit

Background and objectives: effective and sensitive methods for the detection *E. multilocularis* eggs in faecal samples of final hosts are crucial for the prevention and control of echinococcosis. Little is known about the suitability of commercial test kits for isolating DNA of *E. multilocularis* from fox faeces or soil.
We therefore assessed four different test kits: QIAamp Fast DNA Stool Mini Kit (Qiagen); FastDNA® SPIN Kit for Soil (MP); ZR Fecal DNA MiniPrep™ (ZymoResearch) and NucleoSpin® Soil Kit (Macherey & Nagel) for DNA isolation from *E. multilocularis* eggs in faecal fox samples.
Materials and methods: for each test kit, *E. multilocularis* negative faecal samples were spiked with calculated numbers of 2 up to 600 *E. multilocularis* eggs, and each egg concentration was tested 10 times in each of the extraction kits. Each extracted DNA was amplified using three PCR protocols: i. conventional PCR (cPCR, Platinum®Taq, Invitrogen), ii. qPCR with the iQ™ supermix (Biorad) and iii. qPCR with the QuantiTect® Multiplex-Mastermix (Qiagen).
Results: statistical analysis of the PCR revealed that the combination of the QIAamp® Fast DNA Stool Mini Kit with the qPCR using the QuantiTect® Multiplex-Mastermix had the highest analytical sensitivity (97%) for the faecal samples spiked with *E. multilocularis* eggs. Conclusion: The results of present study indicate that it is of utmost importance to select suitable DNA extraction kits and PCR methods or reagents to achieve acceptable analytic sensitivity in detection of *E. multilocularis* eggs in fox faecal samples.

Session 9: Free Topics

October 14, 2016
11:00 – 12:30

Room Zehlendorf
Chairs: Martin Beer and Stephanie Thomas

Mycobacterium avium subspecies paratuberculosis in non-human primates

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Keywords: Mycobacterium avium subsp. paratuberculosis; non-human primates; PCR

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of paratuberculosis disease, which mainly occurs in ruminants. The disease is characterized by a degenerative chronic granulomatous inflammation of the intestinal tract and leads to diarrhea, weight loss, reduced reproductive performance and finally death. Hitherto, the etiological relationship between MAP and Crohn's disease in humans still remains unclear. Nevertheless, the clinical picture of Crohn's disease in humans and the Wasting Marmoset Syndrome in marmosets shows clear parallels to paratuberculosis in ruminants.

To investigate the susceptibility of non-human primates to MAP 20 animals (belonging to 7 different species) were examined after necropsy. Gross and histopathological changes were documented. Collected faecal and tissue samples (ileum, ileocecal lymph nodes, bone marrow) were analysed by IS900-based PCRs and culture.

MAP DNA was detectable in the tissue of two animals; the ileum of a cottontop tamarin and the bone marrow of a common marmoset. However, cultivation of MAP failed and pathohistological examinations offered no direct correlation to an infection with MAP. As both NHP suffered from other diseases an asymptomatic infection with MAP was assumed.

Thus, non-human primates are also susceptible to MAP, detailed analysis of MAP-infected non-human primates could promote further understanding of MAP pathogenesis and the implication of MAP in chronic bowel disease.

Co-Adaptation of the Hemagglutinin Due to Increased pH Stability is Essential in H5N1 HPAIV Evolution.

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Keywords: H5N1 HPAIV, hemagglutinin, virulence

Highly pathogenic avian influenza viruses (HPAIV) cause fatal disease in chicken leading to enormous losses in poultry world-wide. Moreover, repeated zoonotic infections have raised serious concerns of a novel pandemic. Previously, we found that, beside the essential polybasic hemagglutinin (HA) cleavage site (HACS), a lethal phenotype in chicken requires additional virulence determinants. In this study, we focused our follow-up search on the HA.

Using the reverse genetics systems from the HPAIV A/Swan/Germany/R65/2006 (H5N1) (R65) and the low-pathogenic strain A/Teal/Germany/WV632/2005 (H5N1), we generated several HA reassortants, HA1/HA2 chimeras and point mutants. Then, we studied the HA activation pH of those viruses and their virulence in chicken.

We found that the R65 HA variants with exchanged HA1 and/or the point mutations S123R or I124T display a notable decrease of activation pH up to one magnitude. In chicken, this pH decrease is paralleled by a considerable reduction of virulence as low as 0%. Among 2899 database H5 HPAIV sequences, we found the single exchanges HA1 123S or 124I at a frequency of 0.6% but in combination in the overwhelming majority of 97.2% of H5 HPAIV, indicating that both amino acid exchanges together at the HA1/HA2 interface form an essential virulence determinant.

Therefore, beside acquisition of a polybasic HACS, the evolution of HPAIV from low-pathogenic precursors requires co-adaptation of the HA.

Two consecutive outbreaks of *Salmonella* Muenchen linked to pig farming in Germany 2013-2014: Is something missing in our regulatory framework?

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Keywords: Salmonella, Outbreaks, Pigs

Background and objectives: In 2013 and 2014, two consecutive outbreaks of *S*. Muenchen took place in the eastern part of Germany with raw pork as the suspected vehicles. Our objectives were to identify the vehicle and the source of the outbreak in 2014 and a possible connection between the two outbreaks.
Materials and methods: We described the outbreak and conducted a cohort study among staff of an affected nursing home. Local food safety authorities tested raw pork products for *S*. Muenchen and traced back positive food specimens. Human, food and environmental isolates were subtyped by PFGE. Finally, we reviewed the legal framework regarding the protection of the consumers against salmonellosis caused by raw pork products.
Results: We identified different raw pork products as outbreak vehicles. Trace-back investigations narrowed the possible source down to 54 pig farms; *S*. Muenchen was detected on 3 of them. One of these farms had already been the suspected source of the 2013 outbreak. *S*. Muenchen isolates from stool of patients (2013 and 2014), raw pork products and surface swabs of the 3 pig farms shared indistinguishable PFGE patterns. This indicates a common

source of both outbreaks in the primary production of pigs. Current European regulations do not make provisions for *Salmonella* control measures on pig farms that have been involved in human disease outbreaks.
Conclusion: In order to prevent future outbreaks, legislators should consider tightening regulations for *Salmonella* control in causative primary production settings.

Joint use of data from science, public health institutions and industry partners: data structures, scientific aspects and legislative challenges in the context of animal health

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Keywords: public-private partnership, secondary data use, animal health indicators

The goal of the project *PPP-InfoS* is to establish a systematic health monitoring in finisher pigs using already existing data and integrate the information to better understand and measure animal health. The innovation of this joint initiative of public and private partners is to overcome the segregated use of health data. To our knowledge this project is one of the first attempts in veterinary science in Germany, which makes use of an overarching data access for animal health. This idea and its realisation implicate new challenges concerning secondary data use, the integration of disparate data from different data owners as well as legal matters.

The presentation will provide further insight regarding these challenges by taking into account the existing guidelines on *Good Practice of Secondary Data Analysis*. Taking routine slaughter check results and data on the use of antibiotic consumption as an example, we will outline the circumstances for a secondary use. This includes presenting available data structures, considerations on different measures how to establish valid health indicators, but also reflections on legal issues.

Analysing and describing the existing data, we found curious forms of data handling with respect to data stewardship, ownership and the actual data use. Specific projects like *PPP-InfoS* help to demonstrate constrains and the need for further action to clarify legal aspects as well as systematic considerations to ensure the quality of secondary data analyses.

SARS-CoV replication is regulated by p53 via interaction of the SARS-Unique Domain and PL^{pro} with E3 ubiquitin ligase RCHY1

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Keywords: SARS-CoV replication, host cell factors, p53

Background and objectives: Replication and pathogenicity of viruses require the utilization of host cell machineries. We seek to identify host protein factors essential for coronavirus replication with the potential of predicting pathogenicities and of prevention.
Materials and methods: Unbiased, high-throughput Y2H screening and several mammalian/biochemical techniques (Split YFP, Mass Spec, Co-IP, Fluorescence-3-Hybrid) were applied to study protein-protein interactions of SARS-CoV with human cDNA expression libraries.
Results: We identified E3 ubiquitin ligase RCHY1 and calcium/calmodulin-dependent protein kinase II delta (CAMK2D) as interaction partners of the SARS-CoV unique domain (SUD) and of PL^{pro} proteases of different coronaviruses (SARS-/MERS-CoV, HCoV-NL63). The interactions augment RCHY1 stability, which ubiquitinates p53 leading to its increased degradation. The relevance of p53 during CoV replication is demonstrated by significantly reduced replication of infectious SARS-CoV (several orders of magnitude) as well as of two SARS-CoV replicons and the mildly pathogenic HCoV-NL63.
Conclusion: For the first time it is shown that the cellular guardian molecule p53 exerts antiviral activity on the replication of CoVs.

Cold-hardiness of the Asian tiger mosquito *Aedes albopictus*, a vector of multiple viral and parasitic pathogens

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Keywords: mosquito-borne diseases, overwintering, vector biology

Background and objectives: The Asian tiger mosquito *Aedes albopictus* is an important threat to public health due to its rapid spread and its well-recognized role as a vector for dengue and other viruses as well as zoonotic parasites. The rapid development of low-temperature phenotypes after introduction to temperate zones has been identified as a key trait for its successful establishment. The aim of the present study is to elucidate the species´ overwintering success in temperate, relatively cool ecoregions.
Methods and Methods: The mosquito eggs produced by adults from different photoregimes (winter *versus* summer) were exposed to 3°C and used for a detailed morphometric analysis at the ultra-structural level. In addition, mosquito eggs were stepwise cooled down to -2°C, exposed for different times and, after stepwise warming, the larval hatching success from the eggs was determined.
Results: Our detailed metric analysis of *Ae. albopictus* eggs reveals different ultrastructural alterations produced by photoperiod (decreased thickness of the dark endochorion) and cold acclimatization (increased thickness of the middle serosal cuticle). Temperatures ≥0°C do not change the larval hatching success from eggs. An exposure to -2°C reduces the larval hatching success to 50% after 9.5 to 63.3 hours.
Conclusion: The gained knowledge improves the estimation of the vector´s overwintering success in temperate regions and thereby the risk assessment of *Ae. albopictus* associated diseases in Europe.

Poster Presentations

Poster Session: Risk assessment, epidemiology and modelling

R01

Differences in species and prevalence for *Rickettsia spp.* in free-ranging ticks and ticks from dogs in animal shelters

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Keywords: ticks, Rickettsia spp., dogs

Dogs are important companions of humans. Meanwhile they have been suspected to present a risk factor for infection with tick-borne zoonoses. This study aimed to identify differences in tick species and their prevalences for *Rickettsia spp.* in ticks collected from the vegetation and ticks collected from dogs in animal shelters.
Ticks were collected in Southwestern France with Atlantic to Mediterranean climate and in Eastern France and Northern Switzerland with a continental climate. Subsequently ticks and embodied *Rickettsia* were identified.
Ticks from dogs of sites in Southwestern France were mainly identified as *Rhipicephalus spp.* and some as *Ixodes spp*. Flagged ticks consisted almost completely of *Ixodes spp.* In the continental zone, *Ixodes spp*. was predominant in tick species from both collecting methods, showing differences in the species of *Ixodes*. While ticks infestation with *Rickettsia spp*. was almost equal in those from animal shelters and free-ranging ticks at sites in Northern Switzerland and Eastern France, the prevalence for rickettsial bacteria in ticks from animal shelters in Southwestern France was twice as high as in flagged ticks.
As tick species are different in their vector competence for rickettsial species, the risk for human health is depending on locally occurring tick species. In some areas differences in prevalence for *Rickettsia spp*. can lead to an elevated risk for humans to get infected when bitten by a tick in a surrounding characterized by dogs.

R02

Seroprevalence of antropo-zoonotic diseases in two German duck farms – a prospective follow-up study

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Keywords: duck farm workers, Influenza A/B, Parainfluenza 1-3

Duck farm workers and slaughterers bear an increased risk to become infected by pathogens colonizing or infecting the guts and airways of ducks. To estimate this risk, we investigated the sero-prevalence of Influenza A/B, Parainfluenza 1-3, Newcastle-Disease-Virus (NDV), *Campylobacter*, and *Chlamydophilia psittaci* antibodies in two cohorts of duck farm workers and slaughterers in comparison to non-exposed workers. Serum samples were collected in 2004, 2007 and 2010.

The sero-prevalence of the mentioned pathogens was surveyed using standardized ELISA, immunoblot, immunofluorescence, or haemagglutination inhibition assays.

Influenza A/B and Parainfluenza 1-3 sero-prevalence was nearly 100%, and corresponded to the situation that we found in the general population of similar age. Surprisingly, some serum samples were tested positive for NDV but there was no association with exposure, thus it was likely that there were cross-reactions to other paramyxoviruses. The exposed cohort demonstrated an anti-*Campylobacter* IgA seroprevalence of 4.17% in 2004, 5.71% in 2007 and 0.00% in 2010 and an IgG seroprevalence of 8.33% in 2004, 0.00% in 2007, and 4.29% in 2010. There was significantly high sero-prevalence of 27.7% for *C. psittaci*-specific antibodies in exposed slaughterers but 0.0% in stable workers.

In conclusion, the present study shows that there is need to implement health safety mechanisms to prevent *Campylobacter* and *C. psittaci* transmission in duck farms and slaughterhouses.

R03

Crimean-Congo Hemorrhagic Fever Virus Infections in Sub-Saharan Africa: Seroprevalence studies in ruminants

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Keywords: Crimean-Congo Hemorrhagic Fever Virus, Africa, Epidemilogy

Crimean-Congo hemorrhagic fever virus (CCHFV) is considered to be one of the major emerging disease threats spreading to and within Europe. Ticks of the genus *Hyalomma* function as vector as well as natural reservoir of CCHFV. Domestic ruminants and wildlife animals play a crucial role in the life cycle of the ticks, and in the transmission and amplification of the virus. Hence, the detection of CCHFV-specific antibodies in ruminants is a good indicator for the presence of a local virus circulation.

In Africa, human CCHF cases are reported regularly from Mauritania and South Africa. Apart from those countries, the real distribution of CCHFV and the infection rates in animals are fairly unknown. Therefore, one aim of a project of the German Ministry of Foreign Affairs was to identify risk areas for CCHFV infections, to establish diagnostic capacities in the partner laboratories, and to raise the awareness for these highly pathogenic viruses in Mauritania, Sierra Leone, Cameroon and the Democratic Republic of Congo. Novel highly sensitive and specific assays were developed and validated to detect CCHFV specific antibodies in ruminants. In addition, two commercially available assays (for testing human serum) were adapted for use in animals. By using these assays it was possible to identify new risk areas and to determine the distribution of CCHFV. This knowledge is crucial for decision makers and public health authorities in deciding on effective countermeasures and outbreak prevention.

R04

In Silico Prediction and Experimental Confirmation of HA Residues Conferring Enhanced Human Receptor Specificity of H5N1 Influenza A Viruses

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Keywords: Candidate pandemic influenza viruses (CPIV), Informational spectrum method (ISM)

Background and objectives: Early recognition of candidate pandemic influenza viruses (CPIV) is of crucial importance for restricting virus transmission and developing appropriate therapeutic and prophylactic strategies including effective vaccines. Often, the pandemic potential of newly emerging IAV is fully recognized once the virus starts to spread efficiently causing serious disease in humans.
Materials and methods: Here, we used a novel phylogenetic algorithm based on the informational spectrum method (ISM) to identify potential CPIV by predicting mutations in the viral hemagglutinin (HA) gene that are likely to affect critical interactions between the HA protein and target cells from bird and human origin, respectively. Predictions were subsequently validated by generating pseudotyped retrovirus particles and genetically engineered IAV containing these mutations and characterizing potential effects on virus entry and replication in cells expressing human and avian IAV receptors, respectively.
Results: The predictions and hypotheses derived from our *in silico* analysis combined with our subsequent experimental studies to validate these predictions are in line with the recent natural evolution of IAV in Egypt. With respect to mutations that may play a role in the adaption of avian IAV to the human receptor the available evidence leads us to suggest that Egyptian H5N1 HPAIV are evolving towards increased human tropism, a prerequisite for increased pandemic potential of these viruses.

Our data suggest that the ISM-based algorithm is suitable to identify CPIV among IAV strains that are circulating in animal hosts and thus may be a new tool for assessing pandemic risks associated with specific strains.

R05

Presence and distribution of Rift Valley fever virus in Mauritania, Sierra Leone, Cameroon and the Democratic Republic of Congo

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Keywords: RVFV, Sub-saharan Africa und sero-epidemiological survey

Rift Valley fever virus (RVFV) is an emerging pathogen causing devastating epidemics throughout Africa and Arabia, affecting both livestock and humans. RVFV can be transmitted by a broad range of mosquitoes, causing multifold clinical manifestations. The virus was first restricted to Sub-Saharan-Africa but eventually spread even to Madagascar, the Arabian Peninsula and Egypt. The introduction of RVFV into Europe and infection of naïve populations is a major concern for agriculture public health. At the moment, no prevalence data are available for many Sub-Saharan African countries and little is known about the virus circulation. Therefore, within the framework of the *"German Partnership Program for Excellence in Biological and Health Security"*, prevalence and distribution data for RVFV in livestock in Mauritania, Sierra Leone, Cameroon and the Democratic Republic of Congo were determined. We implemented a multi-stage serological and molecular analysis of samples from susceptible species (sheep, goats, cattle, camels), whereat a total of 3979 serum samples were analyzed. In the first stage of investigation the general presence of RVFV-specific antibodies was screened with different serological assays. At the second stage positive specimen were surveyed for potential acute infections via IgM specific ELISA and quantitative real-time RT-PCR. We carried out a comparative analysis on the affected species, regional and national distributions and temporal shifts on the basis of the obtained data. Consequently risk areas can now be defined and public protection methods will be implemented.

R06

Bioaerosols from cattle farms

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Keywords: bioaerosols, risk assesment, dairy farm

In dairy farm buildings, the prevalent airborne microorganisms are not well characterized with respect to their quantity and composition. However, identification and quantification of certain bacteria are useful determining morbid causative agents and performing risk assessment. The analysis of antibiotic resistant bacteria could further provide information on air composition and quality.
Bioaerosols were collected in 10 dairy farms using the PGP and the Holbach filtration device with polycarbonate- or gelatine filters, respectively. Samples were prepared for cultivation based (CFU of bacteria, fungi and staphylococci, ESBL, MRSA) and cultivation independent (TCC and 16SrRNA gene cloning and sequencing, specific PCR) approaches.
Measurement of total cell concentration after DAPI staining showed concentrations of 1.7×10^5 - 3.2×10^6 airborne microorganisms/m^3 in the dairy barns. CFU revealed concentrations between 2.8×10^3 - 6.2×10^4 bacteria/m^3, 0 - $6.7\ 10^4$ CFU fungi/m^3 and 3.2×10^2 -2.9×10^4 staphylococcus/m^3. MRSA as well as ESBL bacteria could not be cultivated. The 16S rRNA gene analysis showed a high diversity in bioaerosols from different stables. Furthermore, DNA of *Mycobacterium avium* subsp. *paratuberculosis* was detectable in two out of ten barns.
The detection of certain bacteria from bioaerosols that are able to cause health problems in humans as well as in animals or showing antibiotic resistance could support a proficient risk assessment for dairy barns.

R07

Using the Temperature-Dependence of the Extrinsic Incubation Period for Spatio-Temporal Risk Assessment: A case study for Dengue in Europe

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Keywords: dengue, risk-mapping, climate change

For many viral arthropod-borne diseases, the duration of the Extrinsic Incubation Period (EIP, the interval between virus acquisition and transmission by the vector), is accelerated by increased ambient temperature. Thus, the raising temperatures expected for Europe due to climate change may result in dramatically shortened transmission cycles and an elevated risk of the so far only sporadic cases of local dengue transmissions to turn into more frequent or severe outbreaks. The relationship between temperature and EIP was modelled by fitting a non-linear unimodal function to experimental data from the literature. Projected future temperature data from a regional climate model was used to produce maps of expected EIP duration for Europe. These were then combined with the results from a species distribution model for the vector *Ae. albopictus* in order to identify areas climatically suitable for fast transmission.

The EIP of dengue was detected to generally shorten in Europe in times of climate change, with the climatic suitability for the vector increasing. Thus, the potential for dengue transmission is likely to increase there. Hotspots for dengue transmission were projected for the Mediterranean islands and the coastline along the Mediterranean and Black Sea – areas that are popular destinations for tourists from countries all across Europe.

Based on this, dengue is no longer a mere tropical disease and can expected to become a major threat for Europe in the future.

R08

Immunogenicity and protective efficacy of recombinant Modified Vaccinia virus Ankara candidate vaccines delivering West Nile virus envelope antigens Vaccine

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Keywords: flavivirus, viral vaccine, poxvirus vector

Background and objectives: West Nile Virus (WNV), an emerging mosquito-borne flavivirus, is maintained in an enzootic cycle between mosquitos as vectors and wild birds. WNV can also infect and cause disease in humans and horses and is able to cause neuroinvasive disease with the potential for severe courses. WNV infections increasingly occur in Mediterranean countries with tendency to spread to central and northern Europe. Thus, safe and efficacious vaccines are sought for WNV prophylaxis in humans and animals. Replication-deficient Modified Vaccinia virus Ankara (MVA) can be exploited as versatile viral vector in medical and veterinary vaccine development.
Materials and methods: We have generated and evaluated recombinant MVA delivering the WNV antigens E (envelope) and prM/M (precursor membrane/membrane) and fulfilling the requirements to undergo clinical testing.
Results: Infections of human and equine cell cultures with recombinant MVA demonstrated efficient synthesis and secretion of WNV envelope proteins in mammalian cells non-permissive for MVA replication. Prime-boost immunizations in BALB/c mice induced circulating serum antibodies binding to recombinant WNV E protein and neutralizing WNV in tissue culture infections. Vaccinations in HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout mice mice resulted in the induction and efficient expansion of WNV-specific CD8+ T cells. Moreover, the MVA-WNV candidate vaccines protected C57BL/6 mice against challenge infections with lineage 1 and lineage 2 WNV and induced heterologous neutralizing antibodies.
Conclusion: Recombinant MVA-WNV vector viruses we have developed here merit further development as candidate vaccines for

potential use in humans and our data strongly support their evaluation in other preclinical models.

R09

Quantitative Risk Assessment Using Bayesian Network Analysis

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Keywords: Risk Assessment; Bayesian Modelling

Background and objectives: The implementation of quantitative microbiological risk assessments (QMRA) requires a stringent approach to the uncertainty and variability inherent in the system under review.
Materials and methods: The traditional statistical approach is to convert the relevant parameters –based on empirical data and expert opinion– into probability distributions which are then assembled into a network structure and analyzed through Monte-Carlo simulations. The latter approach is increasingly being supplemented or replaced by the use of Bayesian networks with the aim of optimizing information yield and taking advantage of the specific capabilities of Bayesian statistics to integrate uncertainty and data. First, a network of priori distributions is built that can then be updated to a network of more informative posterior distributions by providing data for one or more nodes of the network. This process can be repeated several times as new information or data become available and thus leading to updated posteriori model results. Another advantage of Bayesian network analysis is the feasibility of upstream tracking of model parameters whereby, for instance, likely initial parameters of high risk scenarios can be determined.
Results: We demonstrate the development of a generic model for an import scenario where uncertainty and variability stem from misclassification when applying an imperfect diagnostic test and stratification of data pools. The model is implemented in R and JAGS.
We illustrate the advantages of the Bayesian network approach for quantitative microbiological risk assessment using an example in which we quantify the risk of Salmonellosis due to contaminated poultry products imported into the European Union via legal and illegal routes from different world regions based on data with high uncertainty.

R10

Prevalence of potential zoonotic *Clostridium difficile* strains in Germany, Indonesia, Ghana and Tanzania

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Keywords: Clostridium difficile, PCR ribotypes, hospital- and community-acquired infections

C. difficile has been isolated as a commensal or pathogen in the intestines of mammals, birds and reptiles, as well as from the environment and from foodstuff. The increasing incidence of *C. difficile* infections (CDI) in the community raises concerns regarding an animal or food-borne transmission as risk factors for public health. Not only elderly patients are a main risk group, for community-acquired infections especially young and relative healthy individuals are known as predisposing factors.
The aim of the study is to assess the spread of potential zoonotic strains in Ghana, Tanzania, Indonesia and Germany. Patients with diarrhea and asymptomatic control individuals were screened for the presence of *C. difficile* in stool samples. Cultured *C. difficile* strains were subtyped using PCR ribotyping, MALDI-TOF MS, detection of toxin genes, determination of spore titers, as well as antibiotic susceptibility testing.
From a total of 1202 human stool samples of 608 patients and 594 healthy individuals, 97 *C. difficile* isolates could be detected. 15 of the 32 PCR ribotypes have been identified to be present at least in one animal species too. Among potential zoonotic strains, the ribotypes 001/72 (25%) and 078, 014/020, 084(CE) (9.3%, respectively) were most prevalent. The majority of strains (77%; 58/75) were toxigenic. Depending on the origin, different distributions of animal associated ribotypes could be observed. Antibiotic susceptibility patterns revealed a high resistance to Moxifloxacin (67%) and Erythromycin (69%) in Germany, followed by Indonesian strains with 31% and 13% of resistance, respectively. A high diversity of total spore titers could be detected, indicating different capacities of the strains for transmission.

R11

State of the Art and Research Challenges in Face of Climate Change and Globalization

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Keywords: Emerging Diseases, Review, Zoonoses

Background and objectives: Evidence suggests that vector borne and zoonotic diseases (VBZDs) are causing increasing health problems in human societies. Counteracting measures to minimize the risks of VBZDs are required, but still knowledge gaps exist. Here, we review the state of art in the current literature on VBZDs aiming at 1) summarizing knowledge on VBZDs and analyzing geographical patterns and temporal trends; 2) indicating emerged risks during the last decade; and 3) carving out challenges and perspectives for research.
Materials and methods: A systematic literature review was carried out via web of science (Thomson Reuters 2016) in all years' time span. Articles that were cited more than 4 times were included, filtered and classified. Classification included features such as study area, first authors' institute, diseases and vectors covered.
Results: Literature (58) on VBZDs displayed an increasing trend by time, with authors from North America and Europe dominating the field of research. Only European researchers contributed studies on all continents. Seventy per cent of the articles were concerning North America and Europe. VBZDs including West Nile Virus, Lyme disease and Leishmaniosis got more attention than others.
Conclusion: Despite of the increasing number of publications on VBZDs, the research efforts showed a biased distribution pattern on study areas and the VBZDs they focused on. To those continents and VBZDs overlooked before, more efforts are in need.

Poster Session: Pathogen-cell interaction

P01

The vRNP-associated multifunctional host factor ANP32B is involved in the induction of IFN-β during influenza A virus infection

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Keywords: Influenza, ANP32B, IFN-β

Influenza A viruses (IAV) cause severe respiratory disease in humans. In the light of recurrent zoonotic transmissions of IAV and the rapid development of resistance to current antiviral drugs, novel strategies for treatment of acute infections are required. To prevent emergence of drug resistant viruses, advanced antiviral approaches target host proteins involved in virus propagation and progression of infection. For this matter, understanding the protein-protein-interaction network between viral and host proteins is key. Replication of the viral genome and mRNA transcription by the viral ribonucleoprotein complexes (vRNPs) occurs in the cell nucleus. To initiate infection the vRNPs require host protein interactions for efficient polymerase activity. To unravel the vRNP interactome at early times during infection, we applied a proteomics-based approach using recombinant strep-tagged IAV. Purification and MS-analysis of strep-tagged vRNPs from infected A549 cells at four hours post infection revealed binding of the host factor ANP32B, which has recently been suggested to be involved in IAV genome replication. Using NS1-deficient IAV we found an unexpected requirement for ANP32B in the early induction of IFN-β. Knockdown of ANP32B resulted in reduced levels of IFN-β transcripts upon stimulation with poly(I:C) or IAV infection. Consistently, a VSV-based IFN-bioassay confirmed reduced IFN expression. Functional pathway analysis revealed that phosphorylation and nuclear translocation of the transcription factors IRF3 and p65 are not affected by ANP32B knock down. We are currently performing interaction studies of ANP32B in IAV infected cells as well as ChiP-analysis in order to understand how ANP32B is involved in the induction of IFN-ß.

P02

Neutrophil extracellular traps in the *Streptococcus suis* infected cerebrospinal fluid compartment

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Keywords: NETs, meningitis, antimicrobial peptide

Streptococcus (S.) suis infections can lead to suppurative meningitis in pigs and humans, characterized by infiltration of numerous neutrophils. Recently the formation of neutrophil extracellular traps (NETs) has been identified as a fundamental defence mechanism of neutrophils against bacterial infections. NETs consist of nuclear DNA and are able to immobilize and kill entrapped pathogens.
In this study we investigated the interaction of *S. suis* and NETs in the cerebrospinal fluid (CSF) compartment *in vitro* and *in vivo* using immunofluorescence microscopy. Therefore, we analyzed CSF of *S. suis* infected piglets. In addition a model of the human blood-CSF barrier was used, to investigate NET formation after transmigration of neutrophils through this barrier.
Despite the activity of *S. suis* nucleases, NETs with entrapped *S. suis* were detectable in the CSF of infected piglets. *In vitro* results also indicated NET formation and subsequent entrapment of bacteria in the CSF compartment. Interestingly, the human antimicrobial peptide LL-37, which is able to stabilize NETs, was detected *in vitro*. The porcine PR-39, which is homologous to LL-37, was detected in CSF of

piglets with meningitis in high amount compared to piglets without meningitis. Furthermore, we demonstrated for the first time that PR-39 is localized in NETs *in vivo*. These data suggested that NETs may be stabilized by antimicrobial peptides against bacterial nuclease degradation in the CSF compartment.

P03

Host range restriction of insect-specific flaviviruses occurs at several levels of the viral life cycle

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Keywords: insect-specific virus, host range, species barrier

Background and objectives: Insect-specific viruses (ISFs) are closely related to the vertebrate pathogenic flaviviruses but their host range is restricted to insects. The aim of the study was to elucidate at which levels ISFs are blocked with regard to infection of vertebrates.
Materials and methods: Niénokoué virus (NIEV) isolated from mosquitoes was sequenced and phylogenetically analysed. Virus growth was tested in vertebrate and insect cells. A NIEV reverse genetic system was established including a NIEV replicon. Chimeric yellow fever virus (YFV) carrying the envelope proteins of NIEV was generated. The reverse genetic tools were used in electroporation experiments using insect and vertebrate cells.
Results: NIEV is a novel ISF that grew in insect but not in vertebrate cells. Electroporation of a NIEV replicon resulted in RNA replication in insect but not in vertebrate cells, while initial translation of the input replicon RNA occurred in both cell types. Chimeric YFV carrying the envelope proteins of NIEV was obtained via electroporation in insect cells but the recovered viruses did not infect vertebrate cells indicating a block at the level of entry. As the YF/NIEV chimera readily produced infectious particles in insect but not vertebrate cells, restriction is also determined at the level of assembly/release.
Conclusion: Our data suggest that flavivirus host range expansion from insects to vertebrates was a stepwise process that involved overcoming multiple barriers.

P04

Integrin αVβ3 influences Flavivirus replication in mouse cell lines

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Keywords: Integrins, Flavivirus, arbovirus

Background and objectives: Flaviviruses are mosquito borne viruses responsible for large outbreaks around the world. The exact mechanisms underlying Flavivirus entry into the cells are poorly understood and few molecules were characterized as essential for supporting Flavivirus entry and replication. Integrins are heterodimeric transmembrane molecules expressed virtually in all cell lines. Integrins are formed by one alpha and one beta integrin subunit making a possible combination of 25 different integrins which play different roles in cell biology like cell adhesion, signaling and migration. Previous work showed that West Nile virus might use integrins to infect cells efficiently. To better understand the role of integrin in Flavivirus infection we infected mouse cell lines with different Flaviviruses. Materials and methods: Mouse embryonic fibroblast cells (MEFs) lacking the expression of one or more integrin subunits were infected with different Flaviviruses. Using different approaches and techniques we evaluated Flavivirus binding, internalization and replication in MEFs. Results: Flavivirus binding and internalization into the host cell was not affected regardless the presence or absence of integrin. On the other hand, replication was substantially impaired in MEFs lacking αVβ3 integrin. A lower and discrete inhibition was also observed for MEFS lacking the β3 and β1 integrin subunits. Conclusion: Our results clearly show that integrins, in special the αVβ3 integrin, are involved in Flavivirus infection. The exact mechanism which integrins contribute to Flavivirus replication remains unknown and is still under investigation.

P05

The fusion activity of M74 F is increased following co-expression with truncated M74 G proteins compared to the parental protein

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Keywords: African henipavirus, attachment glycoprotein, cell-to-cell fusion

Hendra (HeV) and Nipah virus (NiV), two zoonotic henipaviruses, can lead to severe and fatal infections in humans and other mammals, whereas infected bats do not show any symptoms. Previously it was assumed that the occurrence of henipaviruses is restricted to regions of South East Asia (NiV) and Australia (HeV). In 2009, viral RNA of a novel henipavirus (BatPV/Eid_hel/GH-M74a/GHA/2009 (M74)) has been detected in the spleen of an African flying fox of the species *Eidolon helvum*. So far, the zoonotic potential of the African henipavirus M74 is unknown.

We recently showed that the ability to mediate cell-to-cell fusion following co-expression of the M74 fusion (F) and attachment glycoprotein (G) is restricted to chiropteran cells and that an inefficient transport of M74 G to the cell surface plays an important role for this restricted fusion activity.

Here, we generated truncated versions of M74 G in which amino acids at the cytoplasmic domain end have been deleted. These proteins were subjected to fusion assays.

In contrast to the parental M74 G, the truncated protein was able to induce cell-to-cell fusion not only in chiropteran, but also in different mammalian cells. However, the expression pattern of the truncated G protein did not differ from the full-length G protein.

Our results suggest that the truncated protein enables a more efficient interaction between F and G which is required for the triggering of the non-fusogenic F protein to transit into the fusion-active form.

P06

Analyses of the infection process of different strains of tick-borne encephalitis virus in a novel system of primary murine cortical stem cells

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Keywords: TBEV, neuropathogenesis, novel cell system

Tick-borne encephalitis virus (TBEV) is able to cause an infectious disease in humans that affects the peripheral (PNS) and central nervous systems (CNS). In Europe and Asia this zoonotic disease has accounted for a continuous augmenting number of encephalitis cases over the last 30 years. However, no curative therapy is yet available. Although humans are considered a dead-end host for TBEV, they can still be infected, either peripherally by the bite of an infected tick or orally by consumption of raw milk from an infected animal. In both cases, virions are capable of primary replication in the PNS, before eventually reaching permissive brain tissue. This can lead to CNS inflammation and meningoencephalitis, potentially resulting in permanent damages of the CNS. To shed more light on the neuropathogenesis of TBEV and to provide an additional option to the so far existing models, we attempt to establish a novel cell system for further understanding of the molecular interplay between TBEV and its host. Therefore primary murine neuronal stem cells (MNSCs) were grown in cell culture and infected with different neurotropic strains of TBEV. Semi-quantitative real time PCR was used to monitor the gene expression of several cellular factors involved in the host response upon viral infection. Further, the intracellular replication of the virus was investigated by means of laser scanning confocal microscopy, in order to characterize and analyse the infection process of MNSCs.

P07

Morphological and functional changes in the well-differentiated airway epithelium after infection by influenza virus

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Keywords: influenza viruses, well-differentiated epithelium, air-liquid interface

The airway epithelium is the primary barrier to infection by respiratory pathogens. The main strategy of influenza viruses (IV) to overcome the defense mechanisms of the host is to infect the epithelial cells. However, the virus-host interactions in the respiratory epithelium during long term IV infection are not well characterized.
We developed an air-liquid interface culture system for differentiated porcine bronchial epithelial cells (PBEC) to study the effect of virus-induced cellular damage and the distribution of sialic acid. Two swine IV of different subtypes were used to characterize the infection of the airway epithelium. Recombinant human IV, R1 and R2, were used to analyze viruses which differ in their sialic acid-binding preference.
Our results show that IV infection results in (i) a dramatic loss of cilia, (ii) the reduction of the epithelial thickness and (iii) impairment of mucociliary clearance of the PBEC. Despite these detrimental effects, the barrier function of the PBEC was retained, because the basal cells differentiate into specialized cells and maintain a functional network of tight junctions.
These results provide an explanation why IV infections usually remain restricted to the respiratory tract. Moreover, changes of morphology and expression pattern in the infected epithelium also provide an approach to study late effects of IV infection, including the regeneration of epithelium and the susceptibility to secondary viral/bacterial infections.

P08

Nafamostat inhibits trypsin-independent spread of influenza A viruses

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Keywords: nafamostat, influenza A virus, type II transmembrane serine proteases

Influenza A viruses (FLUAV) depend on cleavage-activation of their hemagglutinin (HA) protein by host cell proteases to acquire infectivity and the responsible proteases are potential targets for antiviral intervention. Type II transmembrane serine proteases (TTSP) activate FLUAV in vitro and TMPRSS2, a TTSP-family member, was shown to be essential for FLUAV spread in mice. The aim of the present study was to identify cell lines, which endogenously express HA activating TTSPs, as well as serine protease inhibitors, which effectively block HA activation by these enzymes.

We found that certain human oesophagus-derived cell lines (OE19, OE21 and OE33) support trypsin-independent spread of FLUAV and siRNA knock-down demonstrated that TMPRSS2 (OE19 and OE33) and TMPRSS4 (OE21) are responsible for HA activation in these cell lines. Moreover, we found that a serine protease inhibitor, nafamostat, blocks HA cleavage and activation by transfected TTSPs and inhibits FLUAV spread in oesophagus-derived cell lines and precision cut slices of non-human primate lung.

Collectively, we identified cell lines which endogenously express HA-activating TTSPs and discovered nafamostat as a potent inhibitor of HA activation by TTSPs.

Poster Session: Antimicrobial use and resistance

A01

Isolation and characterisation of bacteriocins from Escherichia coli

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Keywords: bacteriocins, Escherichia coli

Background and objectives: Bacterial resistance rates to antibiotics and decontaminants are constantly rising. Bacteriocins represent a possible alternative, as these bacterial proteins lyse strains of the same and closely related species. Until now, mainly bacteriocins from *Escherichia* (*E.*) *coli* strains isolated from human samples have been described. To develop a bacteriocin based decontaminant against pathogenic *E. coli* in food, bacteriocin producer strains were isolated from food and environmental samples. These bacteriocins will now be further characterized and compared to the bacteriocins already described.
Materials and methods: In a spot assay overnight cultures and bacteriocin extracts of 136 *E. coli* strains isolated from food and environmental samples were examined for their lysis profile on eight indicator strains.
Results: A bactericidal effect could be observed in 85 (63 %) of the overnight cultures and 51 % of the bacteriocin extracts. After co-cultivation of the bacteriocin producing isolates with the *E. coli* strain DH5α some were able to lyse a broader range of indicator strains.
Conclusion: Percentage of bacteriocin producing *E. coli* strains from food and environmental samples coincides with the percentage rates found in *E. coli* extracted from human samples. The bacteriocins will now be further characterised and their lytic activity tested against pathogenic *E. coli* strains.

A02

Antimicrobial susceptibility and virulence markers in methicillin-resistant *Staphylococcus aureus* (MRSA) from broilers and turkeys

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Keywords: MRSA, poultry, from farm to fork

Background and objectives: Zoonotic MRSA - mainly belonging to the clonal complex CC398 - are frequently detected in livestock. In this study, MRSA from the farm to fork food chain of broilers and turkeys were characterized and compared.
Materials and methods: MRSA from a representative collection obtained at primary production, at slaughter and at retail from poultry (n=173) within the zoonosis monitoring in Germany were chosen to analyse their virulence and resistance gene content using the *S. aureus* Genotyping Kit (Alere Technologies). Antimicrobial susceptibilities and corresponding minimal inhibitory concentrations (MIC) were determined by microdilution according to CLSI and were interpreted using the epidemiological cut off (ECOFF) values provided by EUCAST.
Results: More than 70% of poultry MRSA were non-wildtype against tetracycline, trimethoprim and macrolide-lincosamide-streptogramins (MIC >ECOFF). Staphylococcal enterotoxin (SE) genes were found in one non-CC398 isolate from turkey meat (*seb;* SE production) and in three MRSA CC398 isolates (*sed, sea*) from broilers and turkeys at slaughter and broiler meat. The enterotoxin gene cluster (*egc*) was only found among non-CC398 MRSA. Similarity analysis based on selected resistance and virulence markers revealed a high clonality of non-CC398 MRSA.
Conclusion: Our results underline that next to the predominant MRSA CC398 mainly MRSA CC9 and CC5 occur as poultry associated clones, which are similar in both poultry species.

A03

NGS-based molecular surveillance of MDR bacteria from swine: the "set-up" approach

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Keywords: pig fattening, ESBL-producing E. coli; MRSA

Background and objectives: Food-producing animals are an important source of zoonotic multidrug-resistant (MDR) bacteria via either direct contact or contaminated livestock products.
For implementation of a next-generation sequencing (NGS)-based molecular surveillance system within the national framework of InfectControl2020 (IRMRESS), methicillin resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase(ESBL)-producing *Escherichia coli* and vancomycin-resistant *Enterococci* (VRE) were collected from porcine faeces.
Materials and methods: Samples were taken in 17 pig husbandries in Germany. Detection and identification of target bacteria were carried out by classical microbiological procedures. VITEK-2 was employed for antimicrobial susceptibility testing. The current collection comprises up to six isolates per sample and per target species.
Results:
To date, the strain collection contains 331 ESBL-*E. coli* and 92 MRSA. VRE were not isolated.
Preliminary results of resistance testing revealed differences in resistance phenotypes (PT) on both farm (max. 19 PT) and animal (max. 5 PT) level.
Conclusion: The finding of different PTs within isolates of one bacterial species obtained from an individual animal underlines the necessity to investigate more than one colony per sample to identify the whole diversity of the target species. The current "set-up" approach for an NGS-based surveillance system is promising for the fields of human and veterinary medicine.

A04

Detection of the *mcr-1* gene in *E. coli* isolates from clinical samples of companion animals

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Keywords: colistin, mcr-1, companion animal

Background and objectives: The plasmid-encoded colistin resistance gene *mcr-1* has been reported to be expressed almost exclusively in *Escherichia coli* from livestock animals. We aimed to find out whether *mcr-1* also occurs among bacteria from cats, dogs and horses.
Materials and methods: *A. baumannii* (n=40), *Acinetobacter* spp. (n=15), *E. coli* (n=582), *Enterobacter* spp. (n=9), *Klebsiella* spp. (n=134), and *Pseudomonas aeruginosa* (n=60), obtained in our diagnostic laboratory from clinical samples of cats (n=203), dogs (n=495) and horses (n=142) in the years 1998-2015 were screened for the presence of the *mcr-1* gene by PCR. Whole genome sequencing was performed on an Illumina MiSeq. Antimicrobial susceptibility was determined with the VITEK2 system. The plasmid location of *mcr-1* was verified by CeuI digestion of whole cell DNA and southern blot hybridisation.
Results: The *mcr-1* gene was solely found in the bacterial species *E. coli*. *Mcr-1* positive *E. coli* were associated with enteritis, cystitis and wound infection in 2 cats and 3 dogs in 2013 and 2014. The isolates revealed MICs of polymyxin B between 4 and 8 mg/L, harboured various ESBL enzymes (CTX-M-1/-15, SHV-12, TEM-123) and were resistant to several antimicrobial substances. The *mcr-1* genes were located on IncX4 and IncHI2 plasmids showing similarities with those recently described for isolates from humans and pigs.
Conclusion: This study shows for the first time that the spread of colistin resistant, *mcr-1*-positive *E. coli* is not limited to livestock animals.

A05

Characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and ESBL gene-carrying plasmids of isolates from bovine mastitis

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Keywords: dairy cattle, co-located resistance, conjugative plasmids

Background and objectives: Bovine mastitis is a relevant disease in dairy cows. The aim of this study was to characterize extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* from bovine mastitis and their ESBL gene-carrying plasmids.
Materials and methods: In total, 878 *E. coli* involved in bovine mastitis were isolated from quarter milk samples. ESBL-producers were identified and characterized by antimicrobial susceptibility testing (AST), sequencing of ESBL genes, XbaI-PFGE, multilocus sequence typing (MLST), phylotyping and transfer experiments. Transformants harbouring ESBL gene-carrying plasmids were characterized by AST, PCRs for co-located antimicrobial resistance genes, PCR-based replicon typing and S1-nuclease PFGE.
Results: Twelve ESBL-producers were found (1.4%). They showed eleven XbaI-patterns, eight MLST types [eg. ST10 (n=3) or ST1431 (n=2)] and belonged to the phylogroups A (n=6), B1 (n=2), B2 (n=1) or D (n=3). ESBL genes $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-14}}$ were found on conjugative plasmids (35-225 kb). Five plasmids carried co-located resistances, eg. to sulphonamides (*sul1*) or tetracycline [*tet*(A)]. Two IncHI2-IncP plasmids shared similar characteristics and originated from related isolates.
Conclusion: The detection of related isolates harbouring similar ESBL gene-carrying plasmids points towards a clonal expansion. Co-located resistance suggests that co-selection of ESBL genes may occur even in the absence of β-lactam antibiotics.

A06

VetMAB: Development and implementation of a web-based advanced training program and resistance monitoring tool for German veterinarians with the objective to reduce the antibiotic use in livestock

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Keywords: antibiotic resistance, training, livestock

Background and objectives: The aim of the project is the development of an interactive e-learning-system for teaching responsible handling and prudent use of antibiotics in livestock. Key aspects are the emergence and selection for antibiotic resistances, specific characteristics of different antibiotic agents and animal specific modules. Furthermore, a database system will be developed that offers the opportunity to monitor individual bacterial resistance patterns from farms supervised by participating veterinarians. Participation is free of charge until the end of the funding period.
Materials and methods: A website informs about the training tools and serves as an information and communication platform. The e-learning modules cover basic knowledge and current topics concerning the use of antibiotics in the field of livestock and are divided in a basic and seven advanced species specific modules. The modules are and have been developed in cooperation with the project partners and academic experts. Noteworthy is the innovative implementation achieved by means of different visual and acoustic features supporting the interactive character.
Results: In total, 892 veterinarians have already registered to participate in the four completed, online available modules (certified continuing education).
Conclusion: The overall interest in veterinarians for advanced training regarding prudent antibiotic use is high, even requesting further approach apart from productive livestock.

A07

Occurrence of *bla*$_{VIM-1}$ containing *Escherichia coli* and *Salmonella* deriving from dust-samples isolated in German chicken-fattening farms

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Keywords: multidrug resistance, carbapenemases, livestock,

Carbapenems are one of the last therapeutic options for the treatment of human infections caused by multidrug-resistant gram-negative bacteria. Until now, the occurrence of carbapenemases in livestock and livestock associated surroundings is rarely reported. In 2011 the first VIM-1 producing *Salmonella* Infantis was isolated from a German chicken-fattening farm. In the present study we investigated more than 500 primarily stored bacterial cultures, isolated in 45 chicken-fattening farms during the years 2011-2013. After a non-selective overnight incubation the bacteria were transferred to selective agar plates. Growing *E. coli* as well as *Salmonella* isolates were investigated for the presence of carbapenemase genes by real-time PCR. The received isolates are currently further investigated by using different phenotypic- as well as genotypic approaches and whole genome sequencing. Beside the already by Fischer et al., 2013 described *Salmonella* Infantis strain (R3), one additional *Salmonella* subspecies I (rough phenotype) as well as two *E. coli* isolates were isolated from the selective agar plates. The real-time PCR based screening indicated the presence of the *bla*$_{VIM-1}$ gene in the newly detected *Salmonella* as well as in one of the two *E. coli* isolates. For the second *E. coli* strain no carbapenemase genes have been detected. Further analyses as well as comparing studies between the VIM-1 producing bacteria deriving from the different farms will certainly give more information about the serious problem of the occurrence of CPE within German livestock-farms. Even if the detection rate of carbapenem resistant *E.*

coli and *Salmonella* was scarce, the topic is of utmost importance and a further spread of such bugs has to be avoided by all means.

A08

Analysis of *Staphylococcus aureus* isolates from wildlife and zoo animals

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Keywords: spa typing, macrorestriction analysis, broth microdilution,

Background and objectives: The aim of this study was to characterize *Staphylococcus aureus* isolates from free-living and zoo animals.
Materials and methods: *S. aureus* isolates (11 from free-living animals, 15 from zoo animals, one from captivity) from routine diagnostics were characterized by *spa* typing, macrorestriction analysis and antimicrobial susceptibility testing. Resistant isolates were tested for the respective resistance genes by PCR.
Results: The characterisation of the isolates revealed 19 different *spa* types (3 novel types, 1 untypeable) and 17 main PFGE patterns with up to one subpattern. Only a few isolates were resistant to selected antimicrobial agents. A single isolate proved to be methicillin-resistant and another five isolates were resistant to penicillin. The β-lactamase gene *blaZ* was detected in the MRSA isolate and four of the penicillin-resistant isolates. Two isolates from free-living animals and one from a zoo animal showed elevated MICs of fluoroquinolones and had amino acid substitutions in GyrA/B and/or GrlA. Tetracycline resistance was detected in two isolates from zoo animals and the isolate from the animal in captivity. In these isolates, the tetracycline resistance genes *tet*(M), *tet*(K) and *tet*(L) were detected.
Conclusion: The isolates showed a high diversity of *spa* types and PFGE patterns. The antimicrobial susceptibility testing revealed only resistance to three classes of antimicrobial agents and none of the isolates was multi-resistant.

A09

E. coli harbouring the Colistin resistance gene *mcr-1* from imported reptiles

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Keywords: mcr-1, colistin resistance, reptiles

Background and objectives: Recently, *mcr-1* was described as the first plasmid-mediated polymyxin resistance gene found worldwide in Enterobacteriaceae isolated from livestock animals and humans. This study was carried out to find out whether imported reptiles might carry antimicrobial resistance genes, particularly the *mcr-1* gene.
Materials and methods: From July 2013 to May 2014 we collected 150 fecal samples from various reptile species and countries during routine border controls at the Frankfurt Airport. Selective media were used to screen for antimicrobial resistant gram-negative bacteria and the minimal inhibitory concentration (MIC) was tested for selected strains and data were interpreted using breakpoints provided by the CLSI. PCR and genome sequencing was performed to determine resistance genes, multi locus sequence types (STs), and serotypes.
Results: Thirty-eight ESBL-producing Enterobacteriaceae were identified. For two $bla_{CTX\text{-}M\text{-}55}$ positive *E. coli* strains (ST117, O11:H4 and ST1011, O86:H51) isolated from lizards (*Takydromus sexlineatus*) from Vietnam the MIC for colistin was >4 mg/L and resistance to several other antimicrobial substances was shown. Both strains harboured the *mcr-1* gene on an IncHI2-plasmid and were associated with insertion sequence element IS*Apl1*, as recently demonstrated for a plasmid from a human patient.
Conclusion: The finding of the *mcr-1* gene in reptiles suggests that selective forces other than antimicrobial use, such as animal transport and environmental factors, might select for such strains.

A10

Prevalence and quantification of ESBL/AmpC-producing *Enterobacteriaceae* in retail seafood in Berlin

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Keywords: ESBL/AmpC producing Enterobacteriaceae, prevalence, seafood

Background and objectives: High contaminaion rates of several foods with Extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase-producing *Enterobacteriaceae* have been reported recently. However, there is limited information on the presence of these microorganisms in seafood. The objectives of this study were to determine the prevalence and the quantitative load of ESBL/AmpC producing *Enterobacteriaceae* in retail seafood in Berlin, Germany.
Materials and methods: A total of 120 shrimp and mussel samples from supermarkets and seafood shops in Berlin were screened from December 2015 to April 2016 for ESBL/AmpC producing *Enterobacteriaceae*. ESBL/AmpC production was tested by disc difusion method and ESBL/AmpC genes were identified using multiplex real time PCR. Direct plating on MacConkey agar supplemented with 1mg/L cefotaxime was used for enumeration of ESBL/AmpC producing *Enterobacteriaceae*.
Results: Overall, ESBL/AmpC producing *Enterobacteriaceae* were detected in 21.7% of the seafood samples with a prevalence of 25% and 18.3% for shrimp and mussels, respectively. Of the samples, 97.5% had the ESBL/AmpC producing *Enterobacteriaceae* counts < 100 CFU/g whereas 2.5% had counts of 100 to 1000 CFU/g. Of the 58 isolates, the most predominant ESBL/AmpC types were AmpC-CIT (20.7%), followed by SHV (12.1%) and CTX-M (8.6%).
Conclusion: Results indicate high prevalences of ESBL/AmpC producing *Enterobacteriaceae* in retail seafood, though the quantitative contamination level is low.

A11

Approach to analyze the exposure to resistant *E. coli* along the food chain

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Keywords: antimicrobial resistance, welfare, environmental exposure

The occurrence of antimicrobial resistant microorganisms in livestock is an increasing challenge (WHO, 2001). The aim of the FP7-EU research project EFFORT is to understand the epidemiology of antimicrobial resistance (AMR) in the food chain as well as to understand the relative contribution of exposure routes of AMR from animals to humans.
The German contribution to the EFFORT project is aimed at two levels of the production chain, at herd level and at slaughterhouse level.
At herd level the relationship between herd health and animal welfare on one hand and the frequency of resistant *E. coli* on the other hand is evaluated by means of a self-developed Herd Health and Welfare Index, including animal-oriented and management-based parameters that can be easily assessed at farm level. In addition the Minimum Inhibitory Concentration (MIC) is determined for the *E. coli* isolated from human and animal fecal samples.
At slaughterhouse level, same as at farm level, the environmental exposure of AMR for sentinel human population is based on the analysis of human fecal as well as environmental samples. Based on laboratory results, individuals with high occupational exposure, e.g. farm workers and individuals with medium occupational exposure, such as slaughterhouse workers are compared and potential risk factors are identified.
Comparing two levels of AMR along the food chain to estimate the risk level of resistant bacteria transmitted from animals to human.

Poster Session: Novel methods, diagnostics and NGS

D01

Monoclonal antibodies for the specific detection of immunoglobulins (Ig) and differentiation of Ig-subclasses from Eidolon helvum and Rousettus aegyptiacus fruit bats

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Keywords: fruit bats, immunoglobulins, diagnostic test

Background and objectives: Fruit bats have moved into the focus of scientific attention, due to their postulated role as competent reservoir species for a variety of highly pathogenic viruses. The analysis of their role in certain disease outbreaks is often hampered by the lack of specific diagnostic tools. To close this gap, we aimed at generating monoclonal antibodies (mabs) for the specific detection of IgG and IgM derived from *Eidolon helvum* and *Rousettus aegyptiacus*.
Materials and methods: IgG and IgM of both fruit bat species were purified by affinity chromatography and used for immunization of Balb/c mice. Screening was performed using sera from bats immunized with bovine serum albumin (BSA). To rule out any unspecific binding effects, all clones were also tested on plates that were coated with ovalbumin instead of BSA.
Results: we were able to identify a panel of mabs that specifically bind to either IgG or IgM derived from *Eidolon helvum* or *Rousettus aegyptiacus*.
Conclusion: These newly generated mabs will considerably improve the diagnostic situation for fruit bat samples by enabling the determination of a humoral immune response in animals that may have been in contact with infectious agents.

D02

Nucleic acid-based multiplex technique for the detection and differentiation of avian influenza A virus subtypes H5, H7 and H9

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Keywords: influenza A, poultry, real-time RT-PCR

Beginning of 2015 an increasing incidence of avian influenza outbreaks in commercial poultry was observed worldwide and highly pathogenic avian influenza virus got once again into the focus of public attention. Influenza A viruses belong to the notifiable animal diseases and express the surface antigens haemagglutinin (H) and neuraminidase (N) which can occur in various variants. Especially subtypes H5, H7, H9 are of high epidemiological importance.
After isolation of RNA by using SureFast® PREP DNA/RNA Virus, detection was carried as "One Step RT-qPCR" system with SureFast® Influenza A H5/H7/H9 (both CONGEN, Berlin). This multiplex PCR test is applicable on a variety of real-time PCR instruments equipped for simultaneous detection of four fluorescence emissions at 522 nm, 553 nm, 610 nm and 670 nm. Moreover, the test contains an internal control RNA (extraction and/or amplification control).
Analysis of 113 influenza A strains belonging to subtypes H5 (n=51), H7 (n=38) and H9 (n=24) showed inclusivity of 100%. Beside the target analytes also non-target sequences were examined (n≥180), whereby an exclusivity value of 100% was determined. Depending on the matrix, processing grade, RNA preparation and RNA content the theoretical detection limit was ≤ 25 RNA-copies.
Variants H5, H7, H9 cause significant economic damage in the affected plants and have a high zoonotic potential. Thus, targeted monitoring of different influenza A virus subtypes is gaining increasingly in importance.

D03

Two alternative DNA extraction methods to improve the detection of *Mycobacterium-tuberculosis*-complex members in cattle and red deer tissue samples

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Keywords: bovine tuberculosis, DNA extraction, mycobacteria

Background and objectives: Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* and *M. caprae* is a zoonotic and notifiable animal disease in Germany. Diagnostic procedure is based on a prescribed protocol published in the German bTB legislation. In this, small sample volumes are used for DNA extraction followed by real-time PCR analyses.
As mycobacteria tend to concentrate in granuloma, PCR testing of only small tissue samples may result in false-negative results. In this study, two DNA extraction methods were developed to process large sample volumes.
Materials and methods: The first extraction method is based on a magnetic capture technique, in which specific capture oligonucleotides were utilized, which are linked to magnetic particles and capture MTC DNA released from 10 to 15 grams of tissue material. In a second approach remaining sediments from the first protocol were processed with a less complex extraction protocol that can be used in daily routine diagnostics. A total of 100 tissue samples from cattle and red deer were analyzed with the developed protocols and results were compared to the prescribed protocol.
Results: The use of larger sample volumes led to a sensitivity increase of MTC DNA detection, which was shown by the decrease of Ct-values. Furthermore, five tissue samples, which were tested negative or equivocal by the official protocol, were detected as positive with the alternative extraction methods.
Conclusion: Both new methods yielded an increase in sensitivities for MTC DNA detection in large sample volumes and improve the official diagnostic protocol.

D04

Fluorescence *in situ* hybridization of *Campylobacter fetus*

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Keywords: Campylobacter fetus, FISH-assay

Background and objective: *Campylobacter fetus* divides up into three subspecies: *C. fetus ssp.* f*etus (Cff)*, *C. fetus* ssp. v*eneralis (Cfv)* and *C. fetus* ssp. *testudinum (Cft)*. Infrequently, all three subspecies are isolated from human blood of patients suffering from *C. fetus*-caused sepsis. Our aim was to develop a FISH-assay that can detect all known *C. fetus* subspecies and is able to distinguish *C. fetus* from other *Campyobacter* and *Arcobacter* in order to perform rapid diagnosis.

Material and methods: For FISH-analysis, two *C. fetus*-specific, one *Campylobacter*-specific, one *Campylobacter*/*Arcobacter*-specific, and an Eubacteria-specific probe were used and a strain collection of 33 *C. fetus* isolates. 20 isolates originated from cattle, five from aborted calfs, two from aborted sheeps, one from a pig and five were isolated from human blood. 21 *Cff*, 11 *Cfv* and 1 *Cft* have been analyzed. We also used 40 isolates of non-*C. fetus* species to test the species specificity of the FISH probes.

Results: The *C. fetus*-probe designed to bind in the 23s rRNA-encoding region was highly specific for *C. fetus*, showed no cross-detection and was able to recognize all *C. fetus* subspecies. The FISH-probe designed to bind the 16s rRNA region recognized also other *Campylobacter* species.

Conclusion: We developed a highly *C. fetus*-specfic FISH-assay based on a 23S rRNA-binding probe that can be used to detect all known *C. fetus* subspecies in abortion materials and blood samples.

D05

Mass-spectrometry-based-Phyloproteomics (MSPP) of *Campylobacter fetus*

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Keywords: Campylobacter fetus, MALDI-TOF, mass spectrometry based phyloproteomics

Background and objective: *Campylobacter fetus* divides up into three known subspecies: *C. fetus ssp. fetus* (*Cff), C. fetus* ssp. v*eneralis (Cfv)* and *C. fetus* ssp. t*estudinum (Cft))* which has been isolated from various reptile species, where it apparently is only a colonizer. Our aim was to establish a good mass spectrometry based phyloproteomics (MSPP) typing scheme to replace standard biochemical and molecular subtyping methods.
Material and methods: 33 *C. fetus* isolates were analyzed using MALDI-TOF-based intact cell mass spectrometry (ICMS) and evaluated to establish a *C. fetus*-MSPP scheme. 20 isolates came from colonized cattle, five from aborted calfs, two from aborted sheeps, one from a pig and five were isolated from human blood. 21 *Cff,* 11 *Cfv* and 1 *Cft* have been analyzed. MLST was used as reference method.
Results: Based on the known genome sequences of three *C. fetus* isolates representing all three subspecies, 15 biomarker masses could be assigned to specific gene loci.
Consequently, we were able to differentiate all three subspecies and the most significant MLST clonal complexes out of nine clonal complexes represented in the isolate collection. A MSPP-based UPGMA-tree was constructed.
Conclusions: The principle of MSPP-typing has been successfully adapted to *C. fetus*. The relevant *C. fetus* subspecies/MLST CC can be discriminated using MSPP. This confirms that MSPP bears a high potential of an easy-to-perform typing method.

D06

Retrospective analysis of a serotype 4b variant (IVb-v1) listeriosis outbreak in Germany by whole genome sequencing

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Keywords: Listeria monocytogenes IVb-v1, outbreak, whole genome sequencing

Background and objectives: In 2010, *Listeria monocytogenes* (Lm) strains of the rare serotype IVb-v1 were isolated from listeriosis patients within an outbreak in Southern Germany. PFGE analysis and epidemiological investigations revealed various fish products as the source of infection. Within our project, human isolates and suspicious food isolates were retrospectively typed by whole-genome sequencing (WGS) in order to verify correct assignment to the outbreak cluster. Furthermore, genetic variability of outbreak clones which persisted in the plant facility over 2 years was investigated.
Materials and methods: Lm isolates from patients (9) and six different fish products sampled in one plant (22) were sequenced by Illumina MiSeq and analysed by cgMLST (SeqSphere+) and for single nucleotide polymorphisms (SNP).
Results: All human and food Lm IVb-v1 isolates shared the same PFGE pattern and were assigned to the cluster type 2944 with no or only one allelic difference by cgMLST. Two IVb-v1 food isolates were detected in 2011 and another one in 2013. More detailed SNP analysis showed no genetic alterations of the outbreak clone e.g. in virulence and resistance determinants after persistence in the plant facility.
Conclusion: WGS allows reliable identification of listeriosis outbreak clusters in patients and helps to trace the source in foods. In the present study, the persistence of outbreak clone in the plant facility did not result in higher genetic variability.

D07

A Validated Method for the Detection of C. difficile in Ground Meat

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Keywords: C. difficile, food, detection

Background and objectives: *C. difficile* is the major cause of nosocomial gastrointestinal infections in Germany with increasing incidence. In 2013, 659 (59%) of 1,122 severe cases reported to the RKI had a fatal outcome. Community-acquired cases are often linked to endemic strains with ribotypes that have been described to also occur in various animal species and food items. A zoonotic transmission is assumed. For Germany, no data regarding *C. difficile* prevalence in food have been published until yet and a standardized detection method for food is missing at the moment, preventing a risk assessment for foodborne CDI.
Materials and methods: A cultural detection method was developed that offers the possibility to screen samples at an early time point using real-time PCR. For validation, ground meat was artificially contaminated with vegetative cells or spores of different *C. difficile* strains and analysed regarding the detection limit, selectivity and reproducibility. The real-time PCR assay was assessed regarding selectivity and sensitivity. Finally, the procedure was evaluated in a ring trial study.
Results: The detection method provides reliable results and isolates within 7 days with preliminary data on the third day after sampling. The limit of detection is 10 spores (cfu) / 25 g ground meat. The method proved to be highly selective and reproducible.
Conclusion: A highly sensitive and specific detection method analysing *C. difficile* contamination of ground meat was developed and validated.

D08

Characterization of plasmid-mediated beta-lactamase CMY-2 in *Escherichia coli* from human and animal origin in Germany

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Keywords: antibiotic resistance, AmpC, NGS

Resistance to third-generation cephalosporins in *Escherichia coli* is mediated by extended-spectrum beta-lactamases or AmpC beta-lactamases. Plasmid-encoded *ampC* genes, e.g. bla_{CMY-2}, occur in ca. 1% and 30% of the cephalosporin-resistant *E. coli* from human and poultry, respectively.

Here we performed whole-genome sequencing of CMY-2-producing *E. coli* isolates from studies of the research project “RESET”.

Genomic DNA of CMY-2-positive *E. coli* from different sources (n=60 human, n=5 diseased pig/chicken, n=1 broiler, n=56 chicken meat, n=7 turkey meat) was extracted and sequenced using the Illumina platform. Reads were assembled by A5 algorithm. Phylogenetic markers, such as multi-locus sequence type and plasmid replicon types were identified.

Results of the first 46 sequenced isolates showed a highly diverse distribution in sequence types (STs) and replicon types. 29 different STs were identified; most prevalent were ST10 (n=5) as well as ST69, ST117 and ST57 (n=3 each). Frequent replicon types were FIB (n=36) and FII (n=23). IncB/O (n=24) were detected mainly in isolates from animal/food origin. Additional beta-lactamase genes (bla_{TEM}, bla_{CTX-M}, bla_{OXA}, bla_{SHV}) were detected in 62% of the isolates and two *E. coli* from chicken and retail chicken meat carried the colistin resistance gene *mcr-1.*

First results show no phylogenetic similarity between isolates of human and animal origin, indicating a more likely plasmid mediated spread of bla_{CMY-2} that has to be investigated further.

D09

Rapid molecular identification of the most common indigenous bats species from Germany

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Keywords: genotyping, bats

Background and objectives: Chiroptera form the second largest order of mammals and compromise alone in Germany 23 species. As bats are important hosts involved in the emergence and spread of i.e. zoonotic viruses, it is necessary to discriminate the species. Traditional taxonomic methods rely on morphological features which require experience and can be challenging for cryptic species or if the specimen is of poor condition. Barcoding as one approach requires sequencing and is time consuming. Therefore, a genetic approach for rapid species identification would be valuable.
Materials and methods: In this study, two mitochondrial loci, cytochrome b (cyt b) and cytochrome c oxidase subunit I (COI) were chosen to develop two multiplex qPCRs for differentiating the three most common bat species in Germany (circa 64% archived bat specimen). The remaining bat species can be identified by sequencing the generated internal controls for cyt B and COI, respectively.
Results: The assay was validated with 1000 species belonging to 21 different species. It was possible to determine the three most common species and to sequence the PCR products of the internal control and thereby identify the remaining species. Moreover, the double-check approach (cyt B and COI) makes the determination more reliable.
Conclusion: This multiplex PCR is suitable to improve the genotyping of German bats and is faster than standard approaches.

Poster Session: New and emerging zoonoses

N01

Monitoring of shrew-borne Seewis hantavirus in Germany

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Keywords: Hantavirus, Seewis virus, shrews

Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) are important zoonotic pathogens for humans causing hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. These diseases are caused by rodent-borne hantaviruses that are transmitted to humans via inhalation of aerosolized virus-contaminated rodent excreta. In recent years, a large number of novel hantaviruses has been discovered in shrews, moles and bats. Seewis virus (SWSV) was initially detected in the Eurasian common shrew (*Sorex araneus*), but was later also detected in other *Sorex* species, in the tundra shrew (*Sorex tundrensis*), Siberian large-toothed shrew (*Sorex daphaenodon*) and pygmy shrew (*Sorex minutus*). After the initial detection of SWSV in Switzerland the virus was also found in Germany, Czech Republic, Slovakia, Hungary, Finland, and Far East-Russia. Within small mammal monitoring projects during 2010-2014, about 700 shrews of different *Sorex* species were collected in four regions of Germany. For RT-PCR screening a novel assay was developed targeting a region of the S segment. Using this assay resulted in the identification of 46 positive samples originating from shrews collected in Mecklenburg-Western Pomerania, Thuringia, and Baden-Wuerttemberg. SWSV RNA was detected mainly in *S. araneus*, but also in a few *S. minutus*, and *S. coronatus* indicating spillover infections. In conclusion, this study confirmed a continuing abundance of SWSV in shrew populations at several sites in Germany. Future studies will be dedicated to understand the potential influence of changes in shrew populations on the prevalence and molecular evolution of SWSV. In addition, the zoonotic potential of SWSV will be investigated by serological analyses in risk groups.

N02

Out of the reservoir: CPXV-infections of bank voles (*Myodes glareolus*)

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Keywords: Cowpox virus, virulence, pathogenicity

The species *Cowpox virus* (CPXV) is endemic in Western Eurasia and belongs to the genus *Orthopoxvirus* in the *Poxviridae* family. Rodents, primarily voles, are suspected to be the major reservoir host species of CPXV. However, spill-over infections to accidental hosts including rats, cats, cattle, zoo animals and humans are reported regularly.

To date little is known about the pathogenesis of CPXV inoculated voles beyond that CPXV ecology within its natural reservoir host is different from that in accidental host species. To overcome this, we infected bank voles (*Myodes glareolus*) experimentally. Different CPXV strains and inoculation routes were used to infect bank voles of various ages. Interestingly, neither virus was shed out over a period of 21 days and nor showed the organs tested any viral load. In addition, the majority of bank voles did not exhibit CPXV-specific antibodies.

Here we report our first results about experimental CPXV-infections of bank voles. We found that the bank voles did not become diseased, which contrasts results from experimentally inoculated field voles (Hoffmann *et al*. J Virol. 2015). Further experiments will clarify which factors are needed for successful CPXV-infections of bank voles.

N03

Toll-like-Receptor-4 is essential for *Arcobacter butzleri* induced colonic and systemic immune responses in gnotobiotic IL-10-/- mice

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Keywords: Arcobacter butzleri, Toll-like Receptor-4, pathogen-host interactions

Background and objectives: *Arcobacter butzleri* have been shown to cause sporadic cases of human gastroenteritis with abdominal pain and acute or prolonged diarrhea. Information about the underlying immunopathological mechanisms of infection, however, is limited. For the first time we investigated the role of Toll-like-Receptor (TLR) -4, the main innate receptor for lipopolysaccharide and lipooligosaccharide of Gram-negative bacteria, in murine *Arcobacter* infection.
Materials and methods: Gnotobiotic TLR-4 IL-10 double deficient (TLR-$4^{-/-}$ IL-$10^{-/-}$) and IL-$10^{-/-}$ control mice were generated by antibiotic treatment and perorally infected with *A. butzleri*.
Results: Until day 16 postinfection TLR-$4^{-/-}$ IL-$10^{-/-}$ and IL-$10^{-/-}$ control mice were stably colonized by *A. butzleri*. Infected IL-$10^{-/-}$ mice lacking TLR-4 displayed less pronounced colonic apoptosis that was accompanied by lower numbers of innate and adaptive immune cells within the colonic mucosa and lamina propria as compared to IL-$10^{-/-}$ controls. Furthermore, large intestinal pro-inflammatory mediators including nitric oxide, TNF, IL-6 and MCP-1 and, remarkably, of systemic pro-inflammatory cytokines such as IFN-γ and IL-12p70 were lower in *A. butzleri* infected TLR-$4^{-/-}$ IL-$10^{-/-}$ compared to IL-$10^{-/-}$ mice.
Conclusion: TLR-4 is involved in mediating *Arcobacter* infection *in vivo*. Further studies are needed to investigate the molecular mechanisms underlying arcobacteriosis in more detail.

N04

Sindbis virus (SINV) in *Aedes vexans* and *Culex pipiens* mosquitoes (Diptera: Culicidae)

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Keywords: Culex and Aedes mosquitoes, Sindbis virus (SINV), vector competence

Background & aims: SINV is an arthropod-borne and zoonotic virus. *C. pipiens* is a main vector of SINV, while *A. vexans* is not competent due to a midgut infection barrier. The study's aim was to determine the susceptibility and growth dynamics of SINV in female *A. vexans* mosquitoes in comparison to *C. pipiens* after bypassing the midgut.
Material & methods: Female *A. vexans* (n=31) and *C. pipiens* (n=31) were anesthetized by cooling and injected intrathoracally with 0.17 µl of a SINV solution with $1.0x10^6$ TCID50/ml. Mosquito samples were taken at day 0,3,5,7,9,14 and 21 post infection (p.i.). For each day viral RNA copies were measured by qPCR targeting nsP1 and the viral titers were determined.
Results: SINV was detected in both mosquito species. At day 0 p.i. the qPCR Ct (cycle threshold) value was between 27 and 36 for *A. vexans* and 30-33 for *C. pipiens*. The lowest qPCR Ct value of SINV, representing the peak of genome copies, was between 17.3 and 19.4 at day 3 p.i. for *C. pipiens* and within the same range for *A. vexans* 6 days later. On day 21 p.i., the Ct values were between 20.5 and 22.5 in both mosquito species. Growth dynamics of SINV titers were similar.
Conclusions: Although *A. vexans* is not competent for SINV, it amplified the virus after intrathoracic injection to similar titers as the competent *C. pipiens*. We are currently comparing the growth dynamics of Chikungunya virus in non-susceptible *Culex* mosquitoes in a similar fashion. These results will also be presented.

N05

Prevalence and characterisation of *Yersinia enterocolitica* in retail seafood

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Keywords: Yersinia enterocolitica, prevalence, seafood

Background and objectives: *Yersinia* (*Y.*) *enterocolitica* is a zoonotic enteropathogen widely distributed in Europe, which can cause acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis. Several studies report a high prevalence of *Y. enterocolitica* in pigs and wild boars. No data are available on the prevalence of *Y. enterocolitica* in seafood.
Materials and methods: Seafood samples were purchased randomly from retail shops in Berlin (09/20015 – 04/2016). *Y. enterocolitica* was isolated by selective cold enrichment (PSB) followed by cultivation on selective agar (CIN). Identification, biotyping and serotyping was performed by mPCR assays.
Results: The prevalence of *Y. enterocolitica* in seafood was 2.7% (6/220). Mussel (2/74), shrimp (1/88) and scallop (3/17) samples were positive for *Y. enterocolitica*. All isolates are non-virulent and 3 isolates could be determined as serotype O:8 while 3 samples showed an unknown serotype.
Conclusion: This study provides the first systematic prevalence study of *Y. enterocolitica* in retail seafood in Germany. Although the prevalence is quite low (2.7%) and all the isolates were characterized as non-virulent strains this study shows that seafood might be a potential source of infection with *Y. enterocolitica*.

N06

Gigante virus isolated from a Central-American *Culex* mosquito tentatively forms a novel *Orthobunyavirus* serogroup

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Keywords: arbovirus, orthobunyavirus, mosquito (max. 3)

Background and objectives: The highly diverse genus *Orthobunyavirus* is known to comprise vector-borne emerging pathogens. Here, we sought to investigate the diversity of orthobunyaviruses in mosquitoes from Central-America.
Materials and methods: Mosquitoes were collected in the Panama Canal region and tested by generic RT-PCR. Full genome sequencing was performed by NGS. Insect and vertebrate cells were used for virus isolation and growth kinetics.
Results: A novel orthobunyavirus named Gigante virus (GIGV) was isolated from *Culex melanoconion sp.* in C6/36 and VeroE6/7 cells. Like orthobunyaviruses GIGV L and M segments encode the RdRp and the glycoprotein precursor protein (GPC), respectively. Distinct to orthobunyaviruses but similar to the closest relative Brazoran virus (BRZV), the putative non-structural protein NSs ORF is preceding the nucleocapsid (N) ORF. Maximal amino acid identities of RdRp, GPC and N were 70%, 57% and 26% to BRZV, respectively, suggesting the identification of a new species and putative new serogroup based on ICTV classification criteria. Virus replication was highest in human- and hamster cells. A lower susceptibility was found in cells derived from birds- and Hispanic cotton rats. In contrast, GIGV did not infect bat cells.
Conclusion: We have identified a novel orthobunyavirus, putatively a new serogroup. Rodents may play a key role in the maintenance cycle. Serological investigations on rodent sera collected in parallel during mosquito sampling will help to elucidate host tropism.

N07

Dynamics and risks of MERS-Coronavirus transmission events on the Arabian Peninsula and Africa

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Keywords: MERS-CoV, Camel-to-human transmission, risk assessment

Dromedaries were found to be a reservoir for Middle East respiratory syndrome coronavirus (MERS-CoV) and are the likely source of primary human infections. To date, it is unclear how MERS-CoV is maintained in camel populations and which factors influence camel-to-human transmission.

To assess the dynamics and risks of MERS-CoV transmission we performed a time course study of antibodies in camels and analysed the seroprevalence of humans in contact with livestock in two different countries (United Arab Emirates and Kenya).

All sera were tested by MERS-CoV S1 ELISA and confirmed by virus neutralization test. We monitored MERS-CoV-specific antibody levels in 11 mother-calf pairs over the course of one year post parturition. In addition, we analysed 1425 sera from dromedaries and 1422 human sera from livestock handlers from UAE and Kenya.

We found that MERS-CoV was potentially maintained in camel herds through the introduction of immunologically naïve calves, which lost their maternal antibodies around 6 months post parturition. An overall high antibody detection rate in camels indicated continuous circulation of MERS-CoV in the camel populations of both sampling regions. 2/300 (0.67%) camel workers in UAE and 2/1122 (0.18%) livestock handlers from Kenya harboured MERS-CoV-specific antibodies, rendering camel-to-human transmission a rare event.

In conclusion, we only found a low frequency of camel-to-human transmission of MERS-CoV despite continuous circulation in camel populations.

N08

Proteomics analysis of infectious microalgae of genus Prototheca- potentially zoonotic but rare and a severe infection associated agent.

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Keywords: Prototheca, proteomics, immunodominant proteins

Background and objectives: Protothecosis is a rare but severe disease of animals and humans caused by colourless microalgae of the genus *Prototheca (P.). P. zopfii,* the most common infective species exists as two genotypes (GT), the non-virulent GT1 and GT2 associated with infection. The objective was to create a genotype-specific reference proteome map and identify immunodominant proteins.
Materials and methods: Cells were cultured, harvested and proteins extracted and separated by two-dimensional electrophoresis (2DE). Mass-spectrometry based protein identification was achieved after determining differentially expressed proteins by fluorescence difference gel electrophoresis that matched with the signals of 2DE-Western blotting using sera from experimentally infected rabbits and naturally infected dogs.
Results: A 2DE reference map with 782 spots was established. 107 proteins were identified as differentially expressed (63 up-regulated in GT1 and 44 in GT2), pointing towards an adaptation for intracellular life style. Among the immunodominant proteins we found several (eg, glyceraldehyde-3-phopshate dehydrogenase) described earlier as antigens of eukaryotic pathogens when expressed on the cell surface.
Conclusion: Proteomic analysis indicated that *Prototheca* possesses infection mechanisms similar to other known eukaryotic pathogens. *Prototheca* genotype-specific pathways will be defined once the genome sequence becomes available.

N09

No evidence of bats as reservoir of Bornaviridae in Germany

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Keywords: Bornaviridae, reservoir, bat

Background and objectives: For a long time, mammalian bornavirus (BoDV) has been considered as the prototype of the family *Bornaviridae* and as the cause of Borna disease, a fatal encephalitis of horses and sheep. However, during the last decade many new bornaviruses have been detected in other species, e.g. birds and reptiles, but also in mammals such as squirrels in which the novel zoonotic variegated squirrel 1 bornavirus (VSBV-1) was recently found. The epidemiology of Borna disease points towards a nature-bound reservoir. The bicolored white-toothed shrew was discovered as first reservoir species of BoDV in several endemic areas. Due to the facts that bats are known as important reservoirs for several viruses and that endogenous bornavirus-like elements were recently found in bat genome, bat samples were tested for the presence of bornaviral RNA or antigen as signs of virus infection.
Materials and methods: 260 brain samples of bats (of the family *Vespertilionidae*) from Germany were tested for bornaviral RNA by panRT-PCR. Additionally, organs including brain, liver, lung, kidney and intestine of 101 bats from Bavaria, an endemic area of Borna disease, and 50 bats from non-endemic areas were tested for bornaviral antigen by immunohistochemistry.
Results: Neither bornaviral RNA nor bornaviral antigen was detected in any of the investigated organ samples.
Conclusion: The present study revealed no evidence for bats as reservoir of *Bornaviridae*. The role of endogenous sequences has to be cleared in further studies.

N10

Genetic diversity of *Bartonella* strains obtained from small mammals and their fleas in Germany

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Keywords: small mammals, Bartonella spp., flea-borne pathogens

Background and objectives: Bartonellae cause zoonotic diseases and are transmitted by arthropods. Rodents are reservoirs for most *Bartonella* spp.. As the knowledge about Bartonella in rodents and their parasitizing fleas is scarce in Germany, this study's objectives were to investigate *Bartonella* spp. in small mammals and their ectoparasites.
Material and methods: Small mammals (nine species) were captured and their fleas collected at three sites in Germany, in 2012 and 2013.
Results: Altogether, 660 spleen samples and 450 fleas (11 species) were investigated for *Bartonella* spp. by PCR targeting the ITS 16S-23S rRNA region with subsequent sequencing. In total, 339 (51.4%) small mammals, 258 (57.3%) fleas were positive for *Bartonella* spp.. Most small mammals were positive for uncultured *Bartonella* sp. (n= 42) followed by *B.* sp. N40 (n= 20) *B. grahamii* (n= 9) and *B. taylorii* (n= 8), *B. doshiae* (n=5) and *Cand*. B. rudakovii (n=2). Likewise most fleas were positive for uncultured *Bartonella* sp. (n= 53) followed by *B. grahamii* (n= 12), *B. taylorii* (n= 9), *B.* sp. N40 (n= 9) and *B. elizabethae* (n= 2).
Conclusion: This study's results suggest that rodents and fleas may be reservoirs and vectors, respectively. Zoonotic *B. grahamii* and *B. elizabethae* were found in rodents and their fleas. Though rodent-associated fleas which bite humans are rarely reported, close contact to wild rodents and their fleas may lead to *Bartonella* infection in humans.

N11

Small mammals as virus reservoirs – distribution of bornavirus antigen in organs of naturally infected squirrels and shrews

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Keywords: Bornaviridae, squirrel, reservoir

Background and objectives: Recently, a novel zoonotic bornavirus (VSBV-1) was discovered in variegated squirrels which caused lethal encephalitis in three squirrel breeders. Knowledge about the organ distribution of new viruses such as VSBV-1 is important to gain insights into ways of shedding and thereby maintenance within the potential reservoir population. This is also of importance to assess the risk of possible transmission routes to other species.
Materials and methods: Presence of viral antigen was analyzed in the nervous system, the respiratory, gastrointestinal, urogenital tract, lymphatic tissues and in the skin of six variegated squirrels, three beautiful squirrels and three bicolored white-toothed shrews. Viral X-protein and phosphoprotein were detected by the use of immunohistochemistry applying cross-reactive polyclonal monospecific antibodies generated against BoDV-1.
Results: Viral antigen was detected not only in nervous tissues, but also in respiratory, gastrointestinal, urogenital tract and skin. In these organs, peripheral nerves, and epithelial and mesenchymal cells could harbor viral antigen. This indicates a broad cell tropism of bornaviruses.
Conclusion: The organ distribution of VSBV-1 points to the capacity of shedding via various routes and resembles closely the spread of BoDV-1 in its reservoir host, the bicolored white-toothed shrew. The presence of VSBV-1 in several organs might be the prerequisite to ensure maintenance in the infected squirrel population but implies also the risk for inter-species transmission.

N12

Genetic diversity of arboviruses in mosquitoes and sandflies from Nepal

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Keywords: Arbovirus, Mosquito, Sandfly

Background and objectives: Various arthropod-borne viruses cause diseases such as dengue, chikungunya and blue tongue in humans and animals in Nepal. However, so far no vector-based surveillance study has been conducted. Here, we sought to investigate the diversity of arboviruses in mosquitoes and sandflies.
Materials and methods: Mosquitoes (n=1800) and sandflies (n=152) were collected in human houses, animal sheds, and natural habitats along an altitudinal transect in Nepal. Samples were tested for flaviviruses, alphaviruses, orthobunyaviruses, phleboviruses, rhabdoviruses and orbiviruses by generic RT-PCR. Virus isolations were done in C6/36 and Vero E6 cells.
Results: We identified 5 flavi-like viruses, 4 phlebo-like viruses, 1 rhabdo-like virus and blue tongue virus in mosquitoes and sandflies based on phylogenetic analyses. Furthermore, a novel sandfly-borne phlebovirus which shares 76% pairwise nucleotide sequence similarity to Rift Valley fever virus was discovered. So far, 31% of the pools (n=131) showed cytopathic effects in cell culture. Cell culture supernatants were tested negative by generic PCR assays suggesting the isolation of previously unknown viruses. Deep sequencing of virus isolates is currently ongoing.
Conclusion: Our findings reveal the presence of a great diversity of novel arthropod-associated viruses in Nepalese mosquitoes and sandflies. Further investigation is needed to measure their impact on human and animal health.
Conclusion: These findings provide new insights into the molecular epidemiology, ecology, and prevalence of astroviruses in European bat populations.

N13

Optimization of the cultural detection of *Yersinia pseudotuberculosis*

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Keywords: Yersinia pseudotuberculosis, Wild boar, Isolation

Yersiniosis caused by *Y. enterocolitica* and *Y. pseudotuberculosis* is the third most common bacterial enteritis in European countries. Infections of humans mainly occur by the consumption of contaminated food (pork, vegetables). In this study the commonly used method for the cultural detection of *Y. pseudotuberculosis* was analysed. This species is less frequently isolated than *Y. enterocolitica* and may hence be underrepresented. The existing DIN/ISO10273 method for the detection of pathogenic *Y. enterocolitica* in food and animal feeding stuffs contains no specific methodology for the cultural detection of *Y. pseudotuberculosis*. Consequently there is currently no validated method available for the cultural detection of this species. Aim of this study was to optimize the cultural detection of *Y. pseudotuberculosis* and to apply the method for a prevalence study with wild boars in North-East Germany. Wild boars are suspected to be a reservoir for *Y. pseudotuberculosis*. The DIN/ISO10273 contains a mandatory KOH-treatment of the bacteria, which is a critical step as *Yersiniae* might be inactivated. We therefore varied KOH concentrations and incubation times and included a cold-enrichment step for 7 days in PSB broth. Using the developed protocol, 8 *Y. pseudotuberculosis* strains were isolated from 130 wild boars. In summary, cold-enrichment in PSB-broth was efficient; it also became clear that KOH-treatment is not a suitable step for the cultural detection of *Y. pseudotuberculosis*.

N14

Neutralizing antibodies against MERS Coronavirus in Dromedary Camels, Pakistan, 2012-2015

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Keywords: Middle East respiratory syndrome coronavirus, Pakistan, Dromedary camels

Background: The human pathogenic Middle East respiratory syndrome coronavirus (MERS-CoV) is endemic in dromedary camels in Africa and on the Arabian Peninsula. So far, studies in Kazakhstan and Mongolia reported the absence of MERS-CoV in Bactrian and dromedary camels.
Objectives: Here, we tested dromedaries originating from different herd types and sampled in different regions of Pakistan for MERS-CoV antibodies.
Materials and methods: 565 sera were sampled in 8 districts of Punjab, the eastern province of Pakistan, between 2012 and 2015. Sera were tested using a commercially available Camel Anti-MERS-CoV ELISA. ELISA-reactive sera were subsequently tested in a microneutralization test (NT) for confirmation.
Results: 223 of 565 sera (39.5%) had MERS-CoV neutralizing antibodies. We found 54.4% of nomadic camels positive for MERS-CoV antibodies, contrasted by 32.1% of semi-nomadic camels, and 48.1% of sedentary camels. MERS-CoV antibody positive camels were found in all study years and in 7 of 8 districts tested. In accordance with previous studies herding type (nomadic) and age (>5 years) were found to be significant risk factors for MERS-CoV infection (p-values <0.0001).
Conclusion: Our findings show that the first infections of Pakistani dromedaries with MERS-CoV can be dated back at least to 2012 and suggest a frequent and continuous circulation in Pakistan.

N15

Comparative investigations for the detection of *Giardia* in young dogs and cats from Germany

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Keywords: IFA, genotyping, zoonotic assemblages

The worldwide occurring intestinal parasite *Giardia duodenalis* is currently divided into two zoonotic and six host-specific assemblages. With regard to the zoonotic potential, a reliable detection of patent infections with *Giardia* in dogs and cats as close companions of humans is of major importance. The present study was performed to compare different methods for the detection of *Giardia* and to identify *Giardia* assemblages of dogs and cats from Germany with a maximum age of two years.

For this purpose, 279 canine and 101 feline faecal samples were screened for *Giardia* coproantigen with enzyme linked immunosorbent assay (ELISA) and were tested for *Giardia* cysts with merthiolate-iodine-formalin concentration (MIFC) technique and direct immunofluorescence test (IFA). DNA was extracted from all cyst-positive samples for multilocus sequence typing targeting the SSU rRNA (SSU), glutamate dehydrogenase (gdh) and triosephosphate isomerase (tpi) gene loci.

Giardia coproantigen was present in 34.4 % of the canine samples and in 22.8 % of the feline samples. *Giardia* cysts were detected in 34.5 % of all ELISA-positive samples with MIFC technique and in 80.7 % with IFA. Host-specific *Giardia* assemblages C, D and F were detected in 89.7 % of infected dogs and cats while assemblage A was detected twice.

According to the results of the study, the IFA is a suitable method for the detection of *Giardia* cysts in dogs and cats. A zoonotic potential arising from infected animals could not be excluded.

N16

Analysis of bacteriophage *phi3* in MRSA CC398

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Keywords: MRSA CC398, host adaptation, bacteriophage phi3

MRSA CC398 is transmitted from livestock to humans and emerges in healthcare settings. The phage *phi3* encoding the human-specific immune-evasion cluster (IEC) associated genes *sak*, *chp* and *scn* constitutes a mobile genetic element, which might confer adaptation of MRSA CC398 to the human host.
For testing this hypothesis, we screened 572 MRSA CC398 isolates from patients of the University Hospital Münster for the presence of phage *phi3* using PCR-based methods. Resistance and virulence determinants of *phi3*-positive isolates were identified using DNA microarray analysis. Whole genome sequencing was performed to further characterize bacteriophage *phi3*.
Among the 572 MRSA CC398 isolates, twelve carried phage *phi3*. The isolates belonged to three different *spa*-types and two IEC-types. Resistance genes *mecA* and *tetK/tetM* were present in all isolates and half of the isolates carried genes for erythromycin resistance. Virulence determinants, including genes encoding toxic shock syndrome toxin, enterotoxins or exfoliative toxins, were not found. All isolates belonged to capsule type 5 and carried the biofilm associated genes *icaA*, *icaC* and *icaD*. Whole genome sequencing demonstrated that phage *phi3* ranged between 40667 bp and 43499 bp.
The low percentage (2.1%) of *phi3*-carrying isolates suggests that presence of this phage and the genes of the IEC are not a prerequisite for transmission of MRSA CC398 to humans and not major factors for colonization of humans with MRSA CC398.

N17

Zika Virus (ZIKV) Infection of Human Neural Progenitor Cells and Skin Fibroblasts

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Keywords: Zika Virus, Tropism, Type I interferon

Background and objectives: ZIKV is a flavivirus transmitted to humans by *aedes aegypti* mosquito bites. Although most infections are asymptomatic, congenital transmission may cause brain development abnormalities and/or microcephaly of the foetus. We investigated and compared ZIKV infection of human iPSC-derived neural progenitors (NPCs) and adult skin fibroblasts from which iPSCs were reprogrammed.

Materials and methods: Confluent monolayers of NPCs and fibroblasts were infected with the MR766 strain by adding equivalents of 10^7 plaque forming unit in 24-well plates. The kinetics of virus production were determined by immunofluorescence and by titration of the infectious virus released in the supernatant on VERO cells. The expression of interferon-β (INF-β) and IFN-stimulated genes (ISGs) was determined by real-time PCR.

Results: In contrast to fibroblasts, NPCs were highly permissive to ZIKV infection. NCP infection was positively correlated to INF-β expression that was upregulated by 40-fold over baseline whereas no evidence of INF-β mRNA induction was observed in fibroblasts exposed to ZIKV. However, ISGs expression was increased in both cell types, although at lower levels in fibroblasts than in NPCs.

Conclusion: ZIKV infection triggers a type-I IFN response proportional to the efficiency of infection suggesting that the poor infection of fibroblasts as compared to NPCs might be explained by a differential expression of putative virus receptors and/or capture co-receptors.

N18

Prevalence of *Arcobacter* spp. in retail seafood in Germany

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Keywords: Arcobacter, seafood, prevalence

Background and objectives: *Arcobacter* is an emerging zoonotic pathogen with a wide range of habitats and hosts. *Arcobacter* (*A.*) *butzleri* and *A. cryaerophilus* have been classified as a serious hazard to human health and consumption of contaminated food and water is considered to be the major transmission route to humans. This study reports the prevalence of *Arcobacter* spp. in retail seafood samples in Germany.
Materials and methods: Samples were purchased from supermarkets and retail shops throughout Berlin and *Arcobacter* spp. isolated by a selective enrichment step followed by a filter method on non-selective agar plates. By mPCR and *rpoB* sequencing *Arcobacter* was verified at genus and species level.
Results: *Arcobacter* spp. were isolated from 16 out of 199 seafood samples. For squids 11.8 % (4/34), mussels 8.8 % (6/68) and shrimps 7.1 % (6/84) of the samples contained *Arcobacter* spp., while no *Arcobacter* were detected in scallop samples (0/13). Most of the isolates belong to the species *A. butzleri* (44 %), followed by *A. cryaerophilus* (37 %), *A. venerupis* (12.5 %) and *A. skirrowii* (6.5 %).
Conclusion: Although other reports of *Arcobacter* spp. in retail seafood are higher, e.g. 17.4 % - 41.1 % in Spain, 22.7 % in Chile and 14.7 % - 21.3 % in India, our data support the potential risk of *Arcobacter* transmission to humans by consumption of seafood samples in Germany.

N19

Evidence for an independent third Usutu virus introduction into Germany

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Keywords: Usutu virus, virus phylogeny, immunohistochemistry

Usutu virus (USUV) is an arbovirus within the genus flavivirus, which was first introduced to Southern Europe approximately twenty years ago causing epizootics among wild and captive birds. USUV was initially discovered in wild birds, mainly Common blackbirds (*Turdus merula*), in the Upper Rhine valley in southwest Germany in 2011 and has not spread much northwards since. Phylogenetic analyses revealed that the still ongoing USUV epidemic is caused by two different USUV strains, USUV-Germany belonging to the USUV Europe 3 lineage and USUV-Bonn belonging to the USUV Africa 3 lineage. The two strains were introduced independently.

In August 2015 a new USUV strain, named USUV-Berlin, was isolated in Vero cells from two carcasses of juvenile Great grey owls (*Strix nebulosa*) kept in the Zoological Garden Berlin, which had suffered from a hyperacute fatal systemic infection. Both owls carried high USUV genome loads. Full-length USUV genomes were determined and phylogenetic analysis demonstrated a close relationship with a Spanish mosquito isolate from 2006. Immunohistochemical antigen detection in organ samples of the owls showed the typical USUV infection patterns. Phylogenetic analyses permitted placement of the owl-derived USUV isolate strains within the different USUV lineages. This is the first time that a separate USUV introduction has been detected so far in the northern part of Germany. According to the phylogenetic analysis, USUV-Berlin belongs to the Africa 2 lineage,

and can thus be distinguished from the other strains circulating in Germany. Repeated findings of different USUV strains suggest more frequent introductions into Central Europe and a higher mobility of this virus than assumed to date.

Poster Session: Free topics

F01

Immunohistochemical localization of Heat shock protein 70 in heat treated protoscoleces of *Echinococcus granulosus*

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Key words: Echinococcus granulosus, protoscoleces, heat shock protein 70

Background and objective: Heat shock protein 70 (hsp70) is expressed nearly in all prokaryotic and eukaryotic cells on exposing to stressful conditions. The protoscoleces (Ps) structures that could be involved in hsp70 expression are poorly understood. The present study was aimed to investigate the localization of hsp70 in heat treated Ps.

Methods: Indirect immunofluorescent antibody technique was used to study localization of hsp70 in Ps exposed to 37℃, 42℃ and 45℃. Expression of hsp70 was induced by incubation of 2500 Ps at 36 ºC, 42 ºC, and 45 Cº for 4 hr.

Results: Ps exposed to 37ºC, showed weak histochemical reactions in the tegument (Tg) as well as on the surface of few calcareous corpuscles (CC), while in Ps exposed to 42ºC and 45ºC, a prominent reactions were observed in the Tg, on the surface of CC, and hooklets region. Cells in the sub-tegumental region and muscular region of the suckers showed no histochemical reactions. The majority of 45 ºC treated Ps were responded to such relatively high treatment by sucker protrusion.

Conclusion: Heat shock protein 70 was found to be expressed in the tegument, hooklets region and surface of calcareous corpuscles of 42ºC and 45ºC treated protoscoleces.

F02

Evaluation of heat shocked protoscoleces antigens in the sero-diagnosis of human cystic echinococcosis

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Keywords: cystic echinococcosis, hydatid disease, heat shock proteins, serodiagnosis

Background and objectives: Diagnosis of cystic echinococcosis is complex and has to be confirmed by the combination of immunological tests and imaging techniques. In this study we induced heat shock proteins expression and their immunoreactivity was assessed by ELISA.
Methods and patients: Sera were collected from 34 hydatid patients, 29 healthy donors and 18 non-hydatid cases. For heat shock response, two batches of 25000 protoscoleces (Ps) were incubated separately at 42 °C and 45 °C for 4 hr. Heat treated and normal Ps were disrupted and the resultant supernatant was divided into two parts, one directly used as source of antigens (PE, PE42 and PE45), whereas the second portion was partially purified on Sephadex G150. The immunorectivety of those antigens as well as hydatid fluid was assessed by ELISA. The cut off value to differentiate positive from negative sera was established by ROC analysis.
Results: Extracts of 42ºC treated Ps resulted in two protein peaks and used as PE42P1 and PE42P2 antigens. For 45°C treated Ps, the chromatography pattern resulted in three protein peaks and used as PE45P1, PE45P2 and PE45P3 antigens. Highest rates of sensitivity, specificity and diagnostic accuracy were detected with PE42P2 (91.2 %) and PE45P2 (91.2 %) and sensitivity of ELISA was consistent for Liver cysts with all antigens.
Conclusion: Hydatid antigens extracted from heat treated Ps, were markedly raised the sensitivity of ELISA to detect anti-hydatid IgG AB.

F03

Joint Action EMERGE: Efficient response to highly dangerous and emerging pathogens at EU level

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Key words: networking, outbreak management

The human population is confronted with emerging and re-emerging infectious pathogens that can cause serious cross-border outbreaks. A recent example is the Ebola outbreak requiring strong diagnostic, clinical, and public health measures in Europe and abroad to get this incident under control. The Joint Action (JA) EMERGE is in compliance with the European policy (Decision No 082/2013/EU) where the need for an efficient, rapid and coordinated response to high threat pathogens is defined. It comprises a European network with about 40 diagnostic laboratories focused on risk group 3 bacteria and risk group 3 and 4 viruses. It will act in a so-called inter-epidemic mode (IEM) which can be activated and switched into an outbreak response mode (ORM) on request by the Health Security Committee to direct all activities to the outbreak management. All work packages have specific tasks in relation to the action modes. This new flexible approach for a JA including interim results will be described here on the National Symposium on Zoonoses Research 2016. EMERGE will provide a platform for establishment and consolidation of a common, coordinated and effective response to infectious disease outbreaks at EU level and abroad.

F04

A Varicella-Zoster Virus-Like Herpesvirus in a Great Ape Species: The First Genomic Sequence of a Varicella Virus Infecting Gorillas

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Keywords: Varicella-zoster virus, gorilla, great ape

Background and objectives: In March 2014, the male gorilla „Demba" of the Münster zoological gardens presented with varicella-like papulovesicular rash on trunk and face. Two other gorillas and a chimpanzee presented with similar symptoms. The varicella-like disease seemed to be caused by the human varicella-zoster-virus (VZV) or by a VZV-like herpesvirus of gorillas.
Materials and methods: With generic PCR, a partial DNA polymerase (DPOL) sequence most similar to, but distinct from, VZV DPOL was detected in samples of the diseased gorillas. With specific PCR, also the chimpanzee was weekly positive. As propagation of the virus in cell culture was unsuccessful, we applied a discovery strategy combining in-solution hybridization capture and next generation sequencing (NGS).Results: A near complete genome sequence was amplified by NGS. It revealed essentially the same gene content and organization as VZV, with an average identity of 85%. The novel virus was tentatively named Gorilla gorilla varicella virus (GgorVV). Multiple alignments and phylogenetic reconstructions showed that GgorVV is the closest relative to VZV. Conclusion: The discovery of a VZV-like herpesvirus in 3 gorillas and a chimpanzee suffering from a varicella-like disease, and its genetic characterization unequivocally shows for the first time that a varicella-associated alphavirus exists in gorillas. Since 2 great ape species were infected, a zoonotic potential of GgorVV cannot be excluded.

F05

Large-scale survey for *Leptospira* and *Rickettsia* species in wild small mammal populations in Germany

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Amongst wildlife species, rodents and other small mammals are considered to be important reservoirs for various zoonotic bacteria. Here, we describe a *Leptospira* and *Rickettsia* study of 4,019 rodents and shrews collected between 2010 and 2014 at forest and grassland habitats in Mecklenburg-Western Pomerania, North Rhine-Westphalia, Thuringia and Baden-Wuerttemberg.

Kidney tissue samples were analyzed for *Leptospira* DNA by a PCR targeting the *lipl32* gene. Using this screening PCR, 524 of 3,950 (13.3%) small mammals were tested positive for *Leptospira*. For 279 *lipl32* PCR-positive samples a partial *secY* gene-specific PCR and sequencing were used to identify *L. kirschneri* (63.0%), *L. interrogans* (28.4%) and *L. borgpetersenii* (8.6%). Multi locus sequence typing resulted in the identification of six different sequence types. The highest prevalence was detected in common and field vole.

Spotted Fever Group *Rickettsia*-specific DNA was detected in 314 of 3,939 (8.0%) ear pinna samples using a real-time *glt*A gene-specific PCR. Typing of the rickettsial positive species was performed by a conventional PCR, targeting the partial outer membrane protein B (*omp*B) gene. The *ompB*-based typing for 76 samples resulted in the identification of *R. helvetica* (90.8%), *R. felis* (7.9%) and *R. raoultii* (1.3%). The highest prevalence was found in mouse species.

In conclusion, leptospiral and rickettsial DNA was detected in small mammals from all four regions without obvious host specificity.

F06

Role of the ciliary activity of the airway epithelium in the virus-host interaction

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Keywords: precision-cut lung slices, ciliary activity, swine influenza viruses

The airway epithelium is equipped with the mucociliary clearance system to prevent the detrimental effect of foreign substances including infection by microorganisms. This defense mechanism is based on the mucins produced by mucus-producing cells and the ciliary activity of specialized epithelial cells that transport the mucus out of the respiratory tract.

We applied precision-cut lung slices as a culture system of differentiated airway epithelial cells to analyze the importance of the ciliary activity for the infection by influenza viruses. Conditions were established that result in reversible ciliastasis. By applying this technique, we are able to compare infection in the presence and absence of ciliary activity. This approach will help to determine how efficient is the ciliary activity in preventing virus infection.

Recently, we have shown that the ciliary activity of PCLS can serve as a virulence marker for the respective virus. This finding was obtained by analyzing swine influenza viruses of the subtypes H1N1, H2N1, and H3N2. Depending on their virulence, they caused partial or complete ciliostasis.

Currently, we are applying this approach to determine whether isolates of the pandemic H1N1 virus recovered from humans and swine show differences in their ciliostatic effect and thus in their virulence properties.

F07

First human case of severe septicemia caused by *Mycoplasma capricolum* subsp. *capricolum*

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Keywords: bacteraemia in human, Mycoplasma capricolum subsp. capricolum, Cape Verde Islands

Infections caused by *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*) are usually associated with respiratory diseases, mastitis and arthritis in small ruminants. This is the first report of a human showing severe clinical symptoms associated with a *Mcc* infection.
In January 2014, *Mycoplasma capricolum* subsp. *capricolum* was exclusively isolated from a 62-year old patient with symptoms of recurrent fever and severe limb pain. Septicemia and meningoencephalitis were diagnosed in association with bilateral asymptomatic pneumonia and pleural effusion. On day six after admission, sinusitis and blepharoconjunctivitis were additionally observed. Bacteria were isolated directly from blood. Cultivation of bacteria from cerebrospinal fluid was negative. The bacterial isolate was identified to be *Mcc* by sequencing of the 16S rRNA locus and partial sequencing of the 23S rRNA gene. No other pathogens were detected from specimens investigated. The symptoms had emerged at the end of a tourist visit to the Cape Verde Islands around the New Year 2014. After the return home to Germany, the patient was hospitalized for three weeks and treated with Doxycyclin and Roxythromycin during that period. At the time of discharge from hospital, the clinical symptoms had disappeared and the patient was fully recovered.
Although it was impossible to identify the source of infection, coincidental contact to small ruminants or consumption of food products from goats during a tourist trip may have played a role.

F08

Discovery of two novel alphaviruses in tropical mosquitoes

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Keywords: alphavirus, mosquito-specific viruses

Background and objectives: Most alphaviruses are transmitted by arthropods and infect a broad range of vertebrate hosts. Eilat virus (EILV) is the only described mosquito-specific alphavirus. The aim of this study was to analyse the genetic diversity of alphaviruses in tropical mosquitoes.
Materials and methods: Mosquitoes were collected in Côte d'Ivoire and Panama. Mosquito pools were screened with a generic alphavirus RT-PCR. Full genomes were sequenced and analysed. Virus growth kinetics were performed in insect and vertebrate cell lines.
Results: In total 9.394 mosquitoes (923 pools) from Côte d'Ivoire and Panama were tested for alphaviruses. One pool from Côte d'Ivoire and one pool from Panama were found positive for alphaviruses, tentatively named Taï forest alphavirus (TALV) and Agua Salud alphavirus (ASAV), respectively. The nonstructural proteins of TALV and ASAV showed maximal amino acid identities of 82% to EILV and 66% to Whataroa virus, respectively. TALV grouped with EILV in phylogenetic analyses and ASAV was placed basal to this clade. ASAV was successfully isolated in insect cells. Vertebrate cells did not support replication of ASAV. Replication in insect cells was blocked at temperatures above 31°C suggesting that ASAV is a mosquito-specific alphavirus.
Conclusion: We found two novel alphaviruses related to the only known mosquito-specific alphavirus EILV. These data suggest that mosquito-specific alphaviruses may be more prevalent than previously expected.

F09

Rescue and rehabilitation of bats in E. Slovakia

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Keywords: bats, rehabilitation, buildings

Background and objectives: Many synanthropic bat species roost inside of human buildings year round. In Slovakian cities, especially prefab houses (blocks of flats) are critical from a human-bats conflicts view point.
Materials and methods: The study was carried out in the years 2009 to 2015. The occurence of bats inside the buildings has been systematically recorded. We collected information provided by householders. Each case was investigated (reaction on public request, field investigation, catching of bats, rehabilitation of bats if needed, analysis of the problem, searching for solutions to avoid direct contact between humans and bats inside the buildings).
The public health risk in connection with potential EBLV infection in bats was studied.
Results: Since 2009 we have rescued 756 bats belonging to 5 species. Dehydration was the most frequent. We recorded 5 cases of bats attacked by dogs or cats. A total of 580 bats were systematically sampled for this study. None of the analysed bats was serologically positive for the presence of lyssavirus-specific antibodies.
Conclusion: Bats used to enter the buildings through openings of ventilation, unsealed holes for antennae cables, elevator shafts, untightnesses in old windows and cracks between individual pannels. In cases of invasions into buildings intervention of trained specialists is needed. The majority of the found cases were caused by self-trapping of bats inside of the buildings.

F10

Enterotoxigenic *Staphylococcus (S.) aureus* in raw prawns and raw milk cheese of small ruminants

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Keywords: staphylococcal enterotoxins, raw milk cheese, raw prawns

Background and objectives: Due to their ability to produce staphylococcal enterotoxins (SE) in food coagulase positive *staphylococci* (CPS), mainly *S. aureus* are among the leading causes of foodborne illness. Aim of this study was to characterize the consumer health risk due to enterotoxigenic CPS in cheese made from raw milk from sheep and goats, and raw prawns in Germany.
Materials and methods: 671 samples of raw milk cheese from sheep or goat (n=289) as well as raw prawns (n=381) were taken at retail. If CPS count exceeded >log4 (cheese) and >log3 (prawns) CFU/g isolates were further characterized by mReal-time PCR (*S. aureus* confirmation), *spa* and MLST typing, and a microarray (Alere Technologies) to detect SE-encoding genes and other virulence and resistance determinants. If genes *sea* – *see* were found, SE production was tested by immunoassay (VIDAS SET2, bioMèrieux).
Results: 16 *S. aureus* were assigned to nine different clonal complexes (13 *spa* types). 14 strains harboured at least one SE encoding gene with *sec* and *sel* being the most common. In-vitro SE production was shown for all 12 *sea-see* harbouring strains. Resistance determinants were rarely detected but *lukF-PV*/*lukM* were present in nine strains and *tst* as well as components of the immune evasion cluster (*sak*, *chp* and *scn*) were found in eight strains.
Conclusion: Findings of enterotoxigenic strains in both food matrices proof the necessity of applying process hygiene criteria for CPS to ensure consumer safety.

F11

Corvids, pigeons and gulls as reservoir of *C. psittaci*

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Keywords: C. psittaci, corvids, chlamydiosis

C. psittaci, infecting primarily birds, is one of the main chlamydial species of veterinary interest. To obtain data on its dissemination in Columbidae, Corvidae and Laridae families presented research was conducted.

Cloacal/fecal swabs from wildfowl (254) were taken mainly as a part of ornithology monitoring or in bird rehabilitation centers in different localizations of Poland. Chlamydiaceae-specific qPCR was used as screening method followed by specific PCRs confirming *C. psittaci* presence. Sequencing of ompA gen and phylogenetic analysis of results was used to classify obtained field isolates.

Chlamydiaceae presence was noted in 8.66% tested cases. Differences in level of positivity were observed between families. The highest value was noted for Corvidae (13.39%) with 10 out of 17 samples confirmed as *C. psittaci.* Both positives from Laridae (4.69%) and one out of three from Columbidae (3.18%) also harbored this species. Analysis of ompA gene revealed that obtained *C. psittaci* sequences formed four distinct clades. Host specificity is clearly visible with pigeon sample belonging to genotype B and black-headed gulls (N=2) to Mat116. Most of Corvidae isolates grouped with Swedish isolate 1192 and one with 50338, but none of them was assigned to any of established genotypes.

Presented research shows considerable level of overall chlamydial prevalence with significant molecular diversity of obtained *C. psittaci* isolates. It should be noted that corvids can be important reservoir of this pathogen.

F12

Birds and deer in zoological garden infected by different *Mycobacterium avium* subsp. *avium* strains in Germany

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Keywords: Nontuberculous mycobacteria, zoo animals, transmission

Members of the species *Mycobacterium* (*M.) avium* belong to the nontuberculous mycobacteria (NTM) and are distributed in the direct and indirect human environment; they have been found in soils and waters worldwide, but they affect also variant animals. *M. avium* subsp. *hominissuis* (MAH) is an opportunistic pathogen for humans, swine and other mammals. *M. avium* subsp. *avium* (MAA) is the causative agent of avian tuberculosis; it was also isolated from humans in countries with close contact between man and poultry. The aim of this study was to find out which Mycobacteria can be isolated from clinically diseased birds and mammalians in an individual zoological garden and if there are epidemiological links.
35 tissue samples originating from 13 diseased animals belonging to 7 bird and 3 mammalian species were investigated. Species and subspecies were identified by specific PCR reactions. Isolates were genotyped by MIRU-VNTR and IS*901*-RFLP-analysis.
MAA could be isolated from 20 samples of 10 individual animals belonging to 6 bird species and deer. Altogether 4 combined MAA genotypes could be detected. One bird isolate consisted of a mixed isolate of MAA and MAH. Genotyping results suggest different infection sources (e.g. free-ranging wild birds), and a putative animal to animal transmission of MAA.
As susceptible zoo animals and the soil of the enclosures could be a potential source of NTM infection for humans, close contact should either be avoided or prompt thorough hygiene measures.

F13

Post-exposure vaccination of rabies in humans in Slovakia

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Keywords: infectious disease, rabies, vaccine

Background and objectives: Rabies is the viral disease of central nervous system of warm-blooded animals, which affect domestic and wildlife animals. Rabies kills in the World nearly 70 000 persons per year with more than 95% of cases originating from infected dog bites. Rabies has globally dissemination on the present.
Materials and methods: We analyzed the Report on zoonoses and zoonotic agents in Slovakia and Annual reports of regional public health authorities in Slovakia in the years 2010 - 2014.
Results: The risk of infection in man after contact with rabid animal or with animal suspected in rabies; year 2010 - count of cases 879 (post-exposure vaccination against rabies 721 cases); year 2011 - count of cases 948 (post-exposure vaccination against rabies 805 cases); year 2012 - count of cases 963 (post-exposure vaccination against rabies 784 cases); year 2013 - count of cases 888 (post-exposure vaccination against rabies 570 cases); year 2014 - count of cases 1010 (post-exposure vaccination against rabies 904 cases).
Conclusion: Oral vaccination effectiveness of foxes is proved by the low incidence of rabies in the Slovak republic. However very important is caution in assessment of the cases animal bites of humans and dangerous contacts between human and animals. The preventive measures like pre-exposure and post-exposure vaccination of humans is an essential factor in the prevention of rabies infection.

F14

Variation of the gene expression profiles of *Campylobacter* spp. in response to heat stress

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Keywords: heat stress, gene expression, Campylobacter

Background and objectives: The high susceptibility of *Campylobacter* to several stressors might depend on the lack of typical stress response mechanisms described for other bacteria. Nevertheless, *Campylobacter* (*C.*) spp. is able to overcome barriers along the food chain. While data for *C. jejuni* stress response mechanisms exists, information for *C. coli* and *C. lari* are sparse. As *C. jejuni* showed prolonged survival at 46°C compared to *C. coli* and *C. lari,* we investigated the gene expression profiles of common heat shock genes of the three species.
Materials and methods: Cultures at late exponential growth phase were harvested and resuspended in fresh medium preconditioned to 46 °C and 37 °C, respectively. Total RNA was extracted after 5, 15, 30, 45 and 60 min of heat shock and cDNA synthesised. Using RT-qPCR the gene expression of several heat shock associated genes was determined.
Results: In comparison, highest regulation of gene expression was detected in *C. coli*, followed by *C. jejuni* and *C. lari*. While in *C. jejuni* a continuous increase of mRNA levels was detected over 60 min, the highest mRNA levels in *C. coli* and *C. lari* were reached after 15 min, remaining at this level until 60 min.
Conclusion: Even though differences in the intensity and the time course of regulation could be determined between the three species the tendency of up and down regulation of orthologs is mostly similar.

F15

Antiviral immunity and the interaction of hantavirus with professional antigen presenting cells

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Keywords: Hantavirus, Disease model, Immunopathology

Background and objectives: Hantaviruses (Bunyaviridae) are rodent-borne pathogens which within the natural host cause asymptomatic and persistent infection. Transmission to humans occurs via the respiratory route and leads to one of two serious illnesses; hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS). Both diseases are characterized by increased vascular permeability and decrease in platelet counts. A humanized mouse model has shown that T cell responses are involved in the development of this disease, but what makes one viral strain apathogenic and another deadly is currently still unclear.
Materials and methods: Data was generated from cell culture, virus titration, qRT-PCR, and by cell sorting and sequencing of a library of cellular knock-outs generated by CRISPR technology and infected with pathogenic or apathogenic hantaviruses.
Results: Many human cell types were identified to be permissive or otherwise react to hantavirus infection, resulting in bystander activation of lymphocytes, release of neutrophil extracellular traps from neutrophils, infection of several types of antigen presenting cell including novel cell types and consequent modulation of T cell function. The prime difference, however, found between apathogenic and pathogenic viruses was that apathogenic viruses were non-infectious to professional antigen presenting cells by a post entry mechanism. Candidate cellular genes restricting the replication of apathogenic and pathogenic strains were identified and are currently being confirmed.
Conclusion: We identify a series of cellular genes that may be responsible for the induction and progression of HFRS in humans

F16

Exposure of piglets with MRSA via the airborne route

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Keywords: MRSA, Aerosol chamber, pig

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged shortly after the introduction of methicillin in 1959 (Robinson and Enright, 2004). Since 2004 MRSA was found to be widespread in food producing animals, particularly in pigs (Voss et al., 2005). Since MRSA was found in barn and ambient air of pig farms (Friese et al., Schulz et al, 2012) an airborne transmission and its relevance for environmental and public health is discussed.
Therefore, one aim of our study is to evaluate the dose for a successful colonization of pigs with MRSA ST398 via the airborne route. Groups of nine MRSA negative piglets each are exposed to MRSA aerosols of different concentrations using an aerosol chamber.
During three weeks after the aerosol exposition the piglets were sampled three times a week by spotting different locations.
In the first group we found only one nasal swab positive after aerosol exposition with MRSA (dose: 10^2 cfu/m^3) whereas all piglets in group two were MRSA positive the day after the exposition with MRSA of a dose of 10^4 cfu/m^3. Over the time the number of positive animals varied and after two weeks all pigs remained MRSA negative. One more dose is under investigation currently.
The two tested doses which are comparable to MRSA concentrations found in farm air do not cause a stable colonization in the piglets after the exposition for 24 hours.

F17

Decontamination of high-containment laboratories using dry-fogging with peracetic acid as alternative procedure to formaldehyde fumigation

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Keywords: peracetic acid, decontamination, high-containment

Background and objectives: Inactivation of pathogens including zoonotic viruses and bacteria is important for work safety and to prevent spread of microbes into the environment. Formaldehyde fumigation is a procedure for room decontamination, however, the carcinogenic potential and a narrow temperature range (23-30°C) for efficient decontamination are disadvantageous. Peracetic acid (PAA) is efficient in inactivation of numerous pathogens at low temperatures and concentrations. However, limited data are available for the effectivity of aerolized PAA. In this study, a new technology is used that generates a finely dispersed dry fog with the aim to establish a protocol that inactivates bacteria and viruses.
Materials and methods: A biological safety cabinet serves as a downsized laboratory with a room volume of 1m^3. Data logger record relative humidity (rH) and temperature prior to and during each fogging trial. The dry fog device aerolizes 1-3 ml/m^3 of a disinfectant solution with 4,5% PAA and 22% H_2O_2. Biological indicators with 10^6 *Geobacillus stearothermophilus* spores are placed at defined locations during each run. Different locations, exposure times, rH and PAA concentrations are tested.
Results: Fifty-one seconds of fogging with 1 ml/m^3 of PAA and an exposition time of 60 minutes resulted in the absence of growth of spores. There was no obvious damage at equipment and surfaces after 16 PAA dry fog runs detectable.
Conclusion: Aerolization of PAA as dry fog inactivates up to 10^6 spores *of Geob. stearothermophilus.* Further trials with this protocol will be performed with viruses and bacteria that may serve as surrogates for zoonotic pathogens.

F18

The ferredoxin redox system in the plastid-like organelle (apicoplast) of *Toxoplasma gondii* is crucial for parasite growth

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Keywords: metabolism, parasite, Toxoplasma gondii

Background and objectives: The only known redox system in the apicoplast consists of ferredoxin $NADP^+$-reductase and its redox partner, ferredoxin (Fd). Fd donates electrons to different essential metabolic pathways such as isoprenoid and fatty acid synthesis (FAS). We analyzed the role of TgFd by genetic depletion.
Results: Using a conditional knock-down (kd) system to deplete TgFd in the apicoplast of tachyzoites after 2 days no TgFd signal could be detected anymore by immunofluorescence. The resulting phenotype was a dramatic growth inhibition of TgFd kd parasites implying that TgFd has essential function(s). Correlative light and electron microscopy on individual plaques did not reveal any obvious ultrastructural changes of the TgFd kd implying that the observed growth phenotype is connected to biochemical rather than structural defects. Apicoplast FAS is required for synthesis of short chain fatty acids. We analyzed TgFd kd using stable isotope-resolved GC/MS. Compared to wt parasites preliminary results show a decrease by 30% in $C_{14:0}$ FA in TgFd kd. Moreover, compromised apicoplast isoprenoid synthesis can indirectly affect parasite mobility. Analyzing blinded samples from motility assays revealed a statistically significant lower (20%) motility of the kd clone compared to the complemented strain.
Conclusion: Our data show that ferredoxin plays a role in different metabolic pathways of the apicoplast and that its depletion leads to almost complete growth arrest in vitro.

F19

Comparison of *Vibrio navarrensis* isolates from veterinary and environmental sources to human pathogenic strains

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Keywords: virulence-associated factors, MLST, hemolysis

Background and objectives: *Vibrio navarrensis* was first isolated from aquatic environments and regarded as an environmental species. However, in 2014, the CDC, Atlanta, identified *V. navarrensis* isolates associated with human illness. Our laboratory obtained isolates from domestic animals after abortions. The aim of the study was to characterize veterinary strains for a pathogenic potential.
Materials and methods: 19 German strains including 10 veterinary isolates were confirmed as *V. navarrensis* by *rpoB* gene sequencing and biochemical assays. The presence of genes encoding virulence-associated factors like capsule or pilus biosynthesis proteins, hemolysins and the type VI secretion system (T6SS) was examined by PCR. Furthermore, the hemolytic activity was investigated. To determine the phylogenetic relationship to clinical *V. navarrensis* strains, MLST analyses were performed.
Results: Nearly all strains possessed genes for capsule and pilus biosynthesis. In addition, all isolates were positive for the majority of hemolysin genes studied and showed hemolytic activity against human erythrocytes. Clear genetic differences were observed for the T6SS which was detected in all veterinary isolates while it seemed to be absent in many environmental isolates. The MLST analyses revealed a close relationship between veterinary and human pathogenic strains
Conclusion: Our study indicates a pathogenic potential of veterinary *V. navarrensis* isolates and re-emphasizes the need for further investigation.

F20

Possibility of oral rabies vaccine potentiation by adjuvants

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Keywords: oral rabies vaccine, squalene, squalene

Mucosal immunization with rabies vaccine is used for regular oral immunization of wild foxes. The objective of this study was to develop squalene (SQE) or squalane (SQA) oil-containing adjuvants suitable for mucosal rabies immunisation.
Rabies vaccines with squalene or squalene adjuvant have been developed. The adjuvanted vaccine was prepared in one step at reduced temperature (37 °C). Effectiveness of adjuvants in combination with live (LRV) and inactivated rabies vaccine, respectively was followed. Randombred white mice were immunised by rabies vaccines in dose 0.1 cm^3 at intramuscular (i.m.) and peroral (p.o.) administration. The level of rabies antibodies on day 30 after immunization was determined by rapid fluorescent focus inhibition-test.
SQE adjuvant caused an increase of the antigenic activity of LRV at i.m. administration of about 1.2-times ($p<0.05$) and at p.o. administration 1.3-times ($p<0.01$). SQA adjuvant did not induce statistically significant changes in antibody formation. When comparing the p.o. and i.m. method of administration significant differences were not observed. SQE was slightly more potent than SQA.
Conclusion: SQE adjuvant raises the oral rabies vaccine effectiveness.

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German Research Platform for Zoonoses

National Symposium on Zoonoses Research 2017

12 – 13 October | Berlin

Steering Committee
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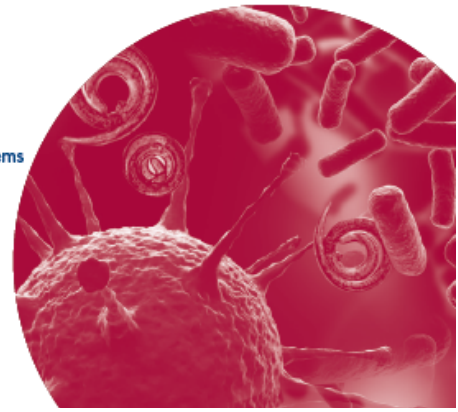

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Thursday, October 13, 2016

08:00	**Registration** (Poster Mounting)
10:00 -12:00	**Plenary Session / Keynotes** *(Ballroom)*
12:00	Lunch and Poster Viewing
14:00 -15:30	**Session 1: Antimicrobial Use and Resistance I** *(Ballroom)*
14:00 -15:30	**Session 2: Pathogen-Cell-Interaction** *(Room Steglitz)*
14:00 -15:30	**Session 3: Risk Assessment, Epidemiology and Modelling** *(Room Zehlendorf)*
15:30	Coffee Break and Poster Viewing
16:00 -17:30	**Big Data Management in Zoonoses Research** *(Ballroom)*
17:30 -19:30	**Mitgliederversammlung der Nationalen Forschungsplattform für Zoonosen** *(Ballroom) (in German)*
19:30	Welcome Reception/Social Dinner

Friday, October 14, 2016

07:30	Young Scientists Breakfast *(Restaurant)*
09:00 -10:30	**Session 4: Antimicrobial Use and Resistance II** *(Ballroom)*
09:00 -10:30	**Session 5: Innate and Adaptive Immune Response** *(Room Steglitz)*
09:00 -10:30	**Session 6: Novel Methods, Diagnostics and NGS** *(Room Zehlendorf)*
10:30	Coffee Break and Poster Viewing
11:00 -12:30	**Session 7: New and Re-Emerging Zoonoses** *(Ballroom)*
11:00 -12:30	**Session 8: Parasites** *(Room Steglitz)*
11:00 -12:30	**Session 9: Free Topics** *(Room Zehlendorf)*
12:30	Lunch and Poster Viewing
14:30 -16:00	**Plenary Session Plenary Session / Keynotes and Farewell** *(Ballroom)*

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