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Welcome Address of the National Research Platform for Zoonoses

Dear colleagues,

it is a great pleasure to welcome you to the National Symposium on Zoonoses Research 2012.

The programme of our meeting addresses the broad spectrum of zoonoses research. Studies concerning epidemiology and risk assessment, immunity and pathogenesis, novel-methods and diagnostics, new and (re-) emerging zoonoses, and antimicrobial use and resistance will be presented. Many thanks to all who submitted abstracts for oral or poster presentations! Renowned speakers complete the programme and will give plenary talks concerning current issues.

We would like to encourage all participants to pay close attention to the poster sessions offering the opportunity to initiate the scientific information exchange and foster interdisciplinary collaborations at the highest level. The best three posters of young researchers will be honored by poster awards.

This year will offer for the first time a young scientists breakfast where young researchers will meet senior scientists face to face to gather advice and inspiration from them about the broad spectrum of career opportunities they may have.

We are looking forward to this formidable scientific meeting and we wish you an enjoyable scientific programme and stay in Berlin.

S. Ludwig	M. H. Groschup	S. C. Semler
(Münster)	(Greifswald – Insel Riems)	(Berlin)

Welcome Notes of the Federal Ministries

Grußwort des Bundesministeriums für Bildung und Forschung zum Nationalen Symposium für Zoonosenforschung 2012

Der Kampf gegen Infektionen erscheint vielen Menschen hierzulande als gewonnen. Bakterien, Viren, Pilze und Parasiten entwickeln jedoch immer neue Strategien, um bisher erfolgreichen Therapien auszuweichen. Zusätzlich schaffen veränderte Lebensumstände wie erhöhte Mobilität, steigende Bevölkerungsdichte oder der Klimawandel neue Nischen und Ausbreitungsgebiete für Krankheitserreger.

Deshalb ist und bleibt der Kampf gegen Infektionserkrankungen ein wichtiger Bestandteil des Aktionsfeldes Volkskrankheiten im „Rahmenprogramm Gesundheitsforschung" der Bundesregierung. In dem abgestimmten Konzept aus thematisch fokussierter und zeitlich befristeter Projektförderung und der dauerhaften Finanzierung bestehender und neuer Institute, bildet die Förderung der Zoonosenforschung einen wichtigen Baustein.

Mit der Einrichtung eines Deutschen Zentrums für Infektionsforschung (DZIF) wurde in diesem Jahr ein zentrales Strukturelement der Forschungsförderung realisiert. Die Deutschen Zentren der Gesundheitsforschung stehen für die Initiative des Bundesministeriums für Bildung und Forschung (BMBF), zur Verbesserung der Lebensqualität durch Erforschung von Volkskrankheiten beizutragen. Neue Ansätze der institutsübergreifenden Zusammenarbeit mit langfristigen Finanzierungszusagen sollen die patientenorientierte Forschung stärken und traditionelle Instituts- und Fächergrenzen systematisch überwinden. Die Deutschen Zentren verfolgen das Ziel den Transfer von Forschungsergebnissen aus dem Labor in die medizinische Versorgung zu beschleunigen. Zoonotische Infektionskrankheiten stehen nicht im direkten Fokus des DZIF, werden jedoch in zahlreichen Schwerpunkten, wie z.B. den „emerging infections", thematisiert.

Wir alle wissen, dass besonders die Menschen in Entwicklungsländern unter Infektionskrankheiten leiden. Es ist deshalb die Verantwortung der Industrienationen zum Kampf gegen diese sogenannten vernachlässigten und armutsassoziierten Erkrankungen beizutragen.
Deutschland stellt sich dieser Verantwortung und investiert weiterhin verstärkt in die Erforschung dieser Krankheiten und ihrer

Bekämpfung. Die Aktivitäten des BMBF sind seit 2011 im Förderkonzept „Vernachlässigte und armutsassoziierte Krankheiten“ zusammengefasst. Ein bedeutendes Element dieses Förderkonzepts ist die substantielle Unterstützung von Produktentwicklungspartnerschaften, kurz PDPs. PDPs werden durch öffentliche und private Geldgeber finanziert. Im Gegenzug werden die Produkte den Betroffenen später zu einem sehr geringen Preis zur Verfügung gestellt. Mit dieser Förderinitiative beschreitet das BMBF für die nächsten vier Jahre einen neuen Weg der internationalen Kooperationen und fördert gezielt die Entwicklung kostengünstiger Präventionsmethoden, Diagnostika und Medikamente.

Diese Fördermöglichkeiten stehen natürlich auch für die Erforschung von Zoonosen offen. Ich freue mich besonders darüber, dass zusätzlich Mittel für Zoonose-Forschungsprojekte für die nächsten drei Jahre reserviert wurden: drei Ministerien - das Bundesministerium für Gesundheit, das Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz und das BMBF - ziehen gemeinsam an einem Strang und stellen die Gelder zur Verfügung. Dies ist eine Chance, die Sie nutzen sollten - stellen Sie für geeignete Pilot- und Querschnittsprojekte Anträge bei der Forschungsplattform für Zoonosen. Die positive Nachricht ist auch der erfolgreichen Arbeit der Zoonosenplattform zu verdanken: Eine Begutachtung durch externe Wissenschaftler hat dies kürzlich bestätigt. Als Ergebnis wird die Zoonosenplattform für weitere drei Jahre vom BMBF gefördert.

Ich freue mich, dass das überaus wichtige Thema „Zoonosenforschung“ mit diesem Symposium ein geeignetes Forum findet und durch die zahlreichen Expertinnen und Experten aus der Wissenschaft vorangetrieben wird.

Dr. Joachim Klein,
Referat Gesundheitsforschung
Bundesministerium für Bildung und Forschung

Grußwort des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz

Zoonosen sind die Tierseuchen der Zukunft! Durch die hohe Mobilität der Menschen, aber auch die Globalisierung der Märkte in Verbindung mit dem Klimawandel und dem raschen Austausch von Waren gewinnen Zoonosen weiter an Bedeutung. Was wäre geschehen, wenn das 2011 erstmals in Deutschland und der Europäischen Union festgestellte Schmallenberg- Virus ein Zoonoseerreger gewesen und auch über stechende Insekten übertragen worden wäre? Welche Möglichkeiten der Bekämpfung, sowohl im Human- als auch im Veterinärbereich hätten wir gehabt? Das Schmallenberg-Virus hat deutlich gemacht, dass jederzeit damit zu rechnen ist, dass ein bei uns nicht heimischer Zoonoseerreger eingeschleppt werden kann. Und das 2006 erstmals in Deutschland festgestellte Blauzungenvirus hat darüber hinaus deutlich gemacht, dass die sogenannten exotischen Erreger nicht notwendigerweise für ihre Replikation die in ihren „Heimatländern" vorhandenen Vektoren benötigen, sondern sich sehr rasch an die heimische Insektenpopulation anpassen. Hier bedarf es der Aufklärung der Mechanismen der Interaktion zwischen einem „neuen" Erreger und einem diesem Erreger fremden Wirt. Diese Thematik steht u.a. auch im Fokus der heutigen Veranstaltung. Vor dem Hintergrund, dass damit zu rechnen sein wird, dass die sogenannten „emerging" oder „reemerging" Zoonoseerreger Vektoren übertragen sein werden, sollte es in jedem Fall Ziel sein, den in Deutschland vorhandenen entomologischen Sachverstand in das Forschungsnetzwerk einzubinden

Beim Auftreten neuer Erreger sind neben der Aufklärung der molekularen Mechanismen auch effiziente Strategien der Bekämpfung gefragt. Grundlage dafür ist einerseits die Kenntnis der Epidemiologie des Erregers, zum anderen aber auch Möglichkeiten einer schnellen und sicheren Diagnose. Auch das werden Sie diskutieren. Insoweit gehen uns die Herausforderungen nicht aus, denn wir müssen jederzeit damit rechnen, dass die theoretischen Grundlagen auch in die Praxis umgesetzt werden müssen!

Nicht zuletzt aus diesem Gesichtspunkt heraus war es wichtig und richtig, dass im Jahr 2006 die drei Bundesministerien, BMBF, BMG und BMELV, der Erforschung der Zoonosen eine größere Aufmerksamkeit geschenkt haben. Für das BMELV, als ein für den gesundheitlichen Verbraucherschutz und die Tiergesundheit gleichermaßen verantwortliches Ressort, eine besondere

Herausforderung. Denn das Thema „Zoonosen“ hat viele Facetten. Dies wird auch an einem weiteren Thema Ihres Symposiums deutlich, widmen Sie sich doch erstmals auch dem „Antibiotikaeinsatz“ und der „Antibiotikaresistenz“, Themen, die gerade auch im Fokus der politischen Diskussion stehen. Seit Jahren ist es ein großes Anliegen des BMELV der Entwicklung von Antibiotikaresistenzen entgegenzuwirken und die Verbreitung von antibiotikaresistenten Bakterien zu verhindern, zumindest aber einzudämmen, denn die Untersuchungen des Bundesinstitutes für Risikobewertung geben Anlass zu der Sorge, dass die Resistenzen bei Tieren zunehmen werden. Diese Erkenntnislage hat dazu geführt, ein Maßnahmenpaket zur Minimierung der Antibiotikaresistenzen mit der Änderung des Arzneimittelgesetzes einzuführen, um eine risikobasierte Überwachung zu ermöglichen.

Das Themenspektrum des diesjährigen Symposiums macht deutlich, dass Sie sich der Herausforderungen bewusst und auch gewillt sind, diese anzunehmen. Ich ermutige Sie ausdrücklich dazu sich zu internationalisieren, vorhandene Netzwerke zu nutzen und ggfl. auszubauen, um durch Ihre Forschung einen Beitrag zur Lösung anstehender Probleme zu leisten.

Ich wünsche Ihrem Symposium einen interessanten und erfolgreichen Verlauf, viele gute Gespräche, in denen innovative Ideen geboren werden, die in interdisziplinäre Forschungsprojekte münden mögen.

Dr. Karin Schwabenbauer
Leiterin der Unterabteilung „Tiergesundheit, Tierschutz“
Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz

Grußwort des Bundesministeriums für Gesundheit

Mit der "Nationalen Forschungsplattform für Zoonosen" im Rahmen der Hightech-Strategie hat die Bundesregierung eine zukunftsorientierte Zusammenarbeit der Human- und Veterinärmedizin geschaffen.
Die "Nationale Forschungsplattform für Zoonosen" hat sich in Deutschland etabliert. Insbesondere das jährlich durch die Plattform ausgerichtete Symposium, die Förderung des Nachwuchses durch fachspezifische Workshops und die Förderung der Pilot- und Querschnittprojekte. Das Bundesministerium für Gesundheit begrüßt es sehr, dass die "Nationale Forschungsplattform Zoonosen" für weitere drei Jahre vom Bundesministerium für Bildung und Forschung gefördert wird.
Für die zweite Förderphase der "Nationalen Forschungsplattform Zoonosen" vergrößert das Bundesministerium für Gesundheit sein Engagement für die Weiterentwicklung der Zoonosenforschung. Für Forschungsprojekte, die dem Forschungsprogramm des Bundesministeriums für Gesundheit entsprechen oder einen Entscheidungshilfebedarf abdecken, kann eine Unterstützung in Aussicht gestellt werden.
Eines der wichtigen Themen, die dieses Jahr im Mittelpunkt des Symposiums stehen, ist von besonderem Interesse für das Bundesministerium für Gesundheit. Resistenzentwicklungen bei Krankheitserregern sind weltweit eine Besorgnis erregende Entwicklung, da die Wirksamkeit der Therapie von Infektionskrankheiten darunter entscheidend leidet.
Das Bundesministerium für Gesundheit hat die "Deutsche Antibiotika-Resistenzstrategie", kurz DART, gemeinsam mit dem Bundesministerium für Ernährung, Verbraucherschutz und Landwirtschaft, dem Bundesministerium für Bildung und Forschung erarbeitet und im November 2008 veröffentlicht. Im humanmedizinischen Teil wurden zehn Ziele formuliert, die bis 2013 umgesetzt werden sollen.
Allen Teilnehmerinnen und Teilnehmern wünsche ich eine gelungene Veranstaltung, einen anregenden wissenschaftlichen Austausch zum Thema Zoonosenforschung und viel Erfolg bei der Umsetzung der gewonnenen Erkenntnisse.

Dr. Antina Ziegelmann
Bundesministerium für Gesundheit

Programme

Thursday 11 October 2012

(Updated 24 September 2012)

09.00 – 16.30 **Registration**

10.30 – 12.30 Plenary Session
(Room Ballsaal)

10.30 – 11.00 **Opening Ceremony**

11.00 – 11.45 **EU surveillance of zoonoses**
Andrea Ammon, Stockholm, Sweden

11.45 – 12.30 **Novel approaches and developments in projecting future patterns of vector-borne diseases**
Carl Beierkuhnlein, Bayreuth, Germany

12.30 – 14.00 Lunch Break

14.00 – 15.30 Session Epidemiology, modelling and risk assessment I
(Room Ballsaal)
Chair: Thomas Müller

Prevalence of antibodies against porcine influenza viruses in humans living in areas with a high pig density of Germany.
J. Lange, A. Krumbholz, R. Dürrwald, M. Walther, T. Müller, D. Kühnel, P. Wutzler, R. Zell

Prevalence of hepatitis E virus (HEV)-specific antibodies in German domestic pigs – a comprehensive serosurvey
A. Krumbholz, S. Joel, R. Dürrwald, R. G. Ulrich, P. Dremsek, M. Walther, P. Wutzler, A. Sauerbrei, R. Zell

Bats worldwide carry hepatitis E-related viruses that form a putative novel genus within the family *Hepeviridae*
J. F. Drexler, A. Seelen, V. M. Corman, A. F. Tateno, V. Cottontail, R. M. Zerbinati, F. Gloza-Rausch, S. M. Klose, Y. Adu-Sarkodie, S. K. Oppong, E. K. V. Kalko, A. Osterman, A. Rasche, A. Adam, M. A. Müller, R. G. Ulrich, E. M. Leroy, A. N. Lukashev, C. Drosten

Zoonotic potential of hepatitis B virus
A. Blasse, M. Kaiser, S. Calvignac-Spencer, K. Merkel, C. Akoua-Koffi, A. V. Adjogoua, C. Boesch, F. H. Leendertz

Influenza viruses inhibit cellular signaling of tyrosine kinases
E. R. Hrincius, S. Liedmann, D. Anhlan, T. Wolff, S. Ludwig and C. Ehrhardt

Immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs) are involved in the antiparasitic defense against *Neospora caninum*
M. Leineweber, K. Spekker, V. Ince, S. Brunder, S. K. Schmidt, G. Schares, W. Däubener

14.00 – 15.30 Session Innate and adaptive immune response

(Room Zehlendorf)
Chair: Martin Beer

Type I interferon antagonistic properties of IAV polymerase proteins regulate pathogenicity
S. Liedmann, E. R. Hrincius, S. Ludwig and C. Ehrhardt

Early protection of balb/c mice against lethal HPAIV H5N1 challenge infection
S. Röhrs, D. Kalthoff, M. Beer

Critical role of perforin-dependent CD8+ T cell immunity for rapid protective vaccination in a murine model for human smallpox
A. Volz, M. Kremer, Y. Suezer, T. Frenz, M. Majzoub, K.-M. Hanschmann, M. H. Lehmann, U. Kalinke, G. Sutter

Campylobacter jejuni **infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses**
A. Fischer, L. M. Haag, B. Otto, U. Grundmann, A. A. Kühl, U. B. Göbel, S. Bereswill, M. M. Heimesaat

Campylobacter jejuni **induces acute enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signaling**
S. Bereswill, L.-M. Haag, A. Fischer, B. Otto, R. Plickert, A. A. Kühl, U. B. Göbel, M. M. Heimesaat

When innate immunity turns inside out: Novel role of phagocyte extracellular traps against zoonotic bacterial infections?
A. Neumann, E. Berends, P. Dersch, V. Nizet, H. Y. Naim, M. von Köckritz-Blickwede

14.00 – 15.30 Session Pathogen-cell interaction I

(Room Steglitz)
Chair: Christian Menge

Study of host-pathogen interactions in *Chlamydia psittaci*- and *Chlamydia abortus*-infected chicken embryos reveals marked differences in dissemination and regulation
M. Braukmann, K. Sachse, I. D. Jacobsen, M. Westermann, C. Menge, H.-P. Saluz, A. Berndt

Characterization of the potential virulence factors CAB063 and CAB821 of *Chlamydia abortus*
V. Forsbach-Birk, M. Wilkat, C. Foddis, U. Simnacher, K. Sachse, D. Longbottom, A. Essig

Modulation of host gene expression upon antimicrobial treatment of persistent *Chlamydia psittaci*
K. Wolf, A. Meinhardt, E. Straube, J. Rödel

Comparative analysis of the six botulinum neurotoxin A subtypes
J. Strotmeier, S. Kull, K. M. Schulz, M. B. Dorner, B. G. Dorner, A. Rummel

Expression of host cell cycle regulators during infections of skeletal muscle cells with *Toxoplasma gondii*
I. J. Swierzy, U. Händel, M. Scharfe, M. Jarek, D. Schlüter, C. G. K. Lüder

***Coxiella burnetii* harbors several anti-apoptotic type IV secretion system substrates**
L. Klingenbeck, R. Eckart, C. Berens, A. Lührmann

15.30 - 16.30 Poster Exhibition

16.30 – 17.30 Plenary Session
(Room Ballsaal)

16.30 – 17.00 **The critical role of science in evidence-based policy – the activities of the World Organisation for Animal Health related to zoonotic disease**
Mary-Kathleen Glynn, Paris, France

17.00 – 17.30 **Antibiotic esistance in animals and humans in the Dutch-German border region**
Alexander Friedrich, Groningen, Netherlands

17.30 – 19.30 **General assembly National Research Platform for Zoonoses (in German)**
- o Annual report
- o Election of the internal advisory board

from 19.30 Get-together

Friday 12 October 2012

08.00 – 09.30 Young scientists breakfast

09.30 – 10.30 Session Novel methods and diagnostics
(Room Zehlendorf)
Chair: Albrecht von Brunn

Functional characterisation of seven botulinum neurotoxin type B subtypes
J. Strotmeier, S. Kirchner, J. Reinke, M. B. Dorner, T. Binz, B. G. Dorner, A. Rummel

Cell culture contaminations – still relevant in the age of technology?
A. Puppe, C. Ulbricht, A. Nitsche, A. Kurth

Validation and establishment of an optimized DNA-microarray system for the non-targeted detection of viruses
B. Abendroth, B. Hoffmann, D. Höper, A. Dastjerdi and M. Beer

Rapid analysis of large diagnostic metagenome datasets – the Schmallenberg virus example
M. Scheuch, D. Höper, M. Beer

09.30 – 10.30 Session Antimicrobial use and resistance
(Room Ballsaal)
Chair: Lothar H. Wieler

Livestock-associated MRSA of the clonal complex 398: Characterization of infection properties
P. Jung, B. Ballhausen, A. Kriegeskorte, L. von Müller, R. Köck, K. Brandt, S. Schwarz, A. Feßler, B. Walther, S. Vincze, G. Peters, M. Herrmann, M. Bischoff and K. Becker

VIM-1 carbapenemase carrying *Escherichia coli* and *Salmonella enterica* from livestock farms.
J. Fischer, I. Rodríguez, S. Schmoger, A. Friese, U. Roesler, R. Helmuth, B. Guerra

Profiling of antibiotic resistance genes in reservoir bacteria isolated from different sources
K. Schwaiger, C. Hölzel, J. Bauer

Replication of human coronaviruses SARS-CoV, HCoV-NL63 and HCoV-229E is inhibited by the drug FK506
Y. Ma-Lauer, J. Carbajo-Lozoya, M. A. Müller, S. Kallies, V. Thiel, C. Drosten and A. von Brunn

09.30 – 10.30 Session Pathogen-cell interaction II

(Room Steglitz)
Chair: Ralph Goethe

Correlation between *Mycobacterium subsp. paratuberculosis* infection and colitis
A. Suwandi, I. Bargen, M. Krey, R. Goethe, M. W. Hornef, S. Weiss

Establishment of molecular methods to detect autochthonous flea species on small mammals and investigation of their associated pathogens
F. Mohr, M. Kiefer, S. Speck, D. Kiefer, S. Eßbauer

Infectivity in peripheral muscle but not in the lymphoreticular system of cattle clinically affected challenged with atypical BSE
A. Balkema-Buschmann, G. Priemer, C. Fast, M. Keller, M. H. Groschup

Ebola virus entry into host cells: Analysis of new receptor candidates
F. Kaup, C. Karsten, K. Gnirss, M. Kiene, A. Krueger, S. Pöhlmann, H. Hofmann

10.30 – 11.30 Poster Exhibition

11.30 – 13.00 Session Pathogen-cell interaction III

(Room Zehlendorf)

Chair: Christian Drosten

Acquisition of new protein domains by SARS-coronavirus: unctional and structural dissection of the SARS-unique domain (SUD)

R. Hilgenfeld, J. Tan, Y. Kusov, C. L. Schmidt, Y. Ma-Lauer, A. von Brunn and J. Lei

The SARS-Coronavirus-host interactome: Identification of cyclophilins as targets for pan-Corona-virus inhibitors

A. von Brunn, Y. Ma-Lauer, S. Pfefferle, J. Carbajo-Lozoya, M. A. Müller, S. Kallies, V. Thiel, R. Hilgenfeld, S. Pöhlmann, F. Weber, G. Herrler, C. Schwegmann-Weßels, H.-J. Thiel and C. Drosten

The influenza virus and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in the human respiratory and gastrointestinal tracts

S. Bertram, A. Heurich, H. Lavender, S. Gierer, S. Danisch, P. Perin, J. M. Lucas, P. S. Nelson, E. J. Soilleux, S. Pöhlmann

A reverse genetics approach to study the determinants for dsRNA-binding and PKR inhibition of the influenza A virus NS1 protein

K. L. Schierhorn, T. Wolff

The combined action of Influenza Virus and *Staphylococcus aureus* Panton-Valentine Leukocidin provokes severe lung epithelium damage

S. Niemann, C. Ehrhardt, E. Medina, K. Warnking, L. Tuchscherr, V. Heitmann, S. Ludwig, G. Peters, B. Löffler

Comparison of glycoprotein-related pathogenicity functions of Lyssaviruses
T. Nolden, J.-P. Teifke, S. Finke

11.30 – 13.00 Session Epidemiology, modelling and risk assessment II

(Room Ballsaal)
Chair: Sandra Eßbauer

Multiple pathogen infections in rodents during a monitoring study in Germany
R. G. Ulrich, N. Kratzmann, S. Schmidt, U. M. Rosenfeld, K. Nöckler, A. Mayer-Scholl, S. Guenther, C. Klotz, T. Aebischer, S. Eßbauer

Antibody prevalence study for human pathogenic viruses in bats and rodents
M. A. Müller, R. G. Ulrich, C. Reusken, J. F. Drexler, E. Lattwein, S. E. Biesold, F. Gloza-Rausch, T. Binger, V. Cottontail, V. M. Corman, K. Fechner, E. K. V. Kalko, S. Oppong, B. M. Kümmerer, J. Schmidt-Chanasit, E. M. Leroy, C. Drosten

Prevalence of antibodies against Tick-Borne Encephalitis Virus in wildlife in Saxony
U. Plessow, R. Lohse, C. Karnath, D. Woll, C. Müller, M. Beer, M. Pfeffer

Diversity of *Bartonella* species in small mammals and their fleas in a recreational area in Leipzig, Germany
C. Silaghi, D. Woll, K. Pfister, M. Pfeffer

Molecular typing of *Toxoplasma gondii* DNA detected in human samples from Germany
D. C. Herrmann, U. Groß, A. Hotop, W. Däubener, K. Spekker, U. Pleyer, O. Liesenfeld, F. J. Conraths, G. Schares

Frequency and characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* from companion animals and horses
C. Ewers, S. Guenther, T. Semmler, T. Janssen, P. A. Kopp, L. Wieler, I. Stamm, A. Bethe

11.30 – 13.00 Session New and re-emerging zoonoses

(Room Steglitz)
Chair: Thomas C. Mettenleiter

***Chlamydia* psittaci inclusion membrane protein IncB associates with host protein Snapin to interact with the host microtubule network**
S. Böcker, A. Heurich, S. Monajembashi, H. P. Saluz, F. Hänel

The novel family *Mesoniviridae* as a potential model of virus emergence
F. Zirkel, A. Kurth, J. Ziebuhr, C. Drosten, S. Junglen

Establishment of a new H1N2 lineage of swine influenza viruses in Germany
J. Lange, M. Groth, A. Krumbholz, M. Platzer, M. Schlegel, R. Zell, R. Dürrwald

Vector competence of German *Culex* species for West Nile virus
M. Leggewie, M. Badusche, J. Börstler, K. Huber, S. Jansen, A. Krüger, E. Tannich, V. Sambri, J. Schmidt-Chanasit, S. Müller

Medical importance of Usutu virus in south-west Germany
L. Allering, G. Hütter, H. Jöst, E. Lattwein, K. Steinhagen, P. Emmerich, S. Günther, J. Schmidt-Chanasit

Susceptibility studies with rabies virus strain isolates in German raccoons (*Procyon lotor*)
A. Vos, S. Finke, C. Freuling, J. P. Teifke, C. Habla, T. Müller

13.00 – 14.30 Lunch Break

14.30 – 16.00 Plenary Session
(Room Ballsaal)

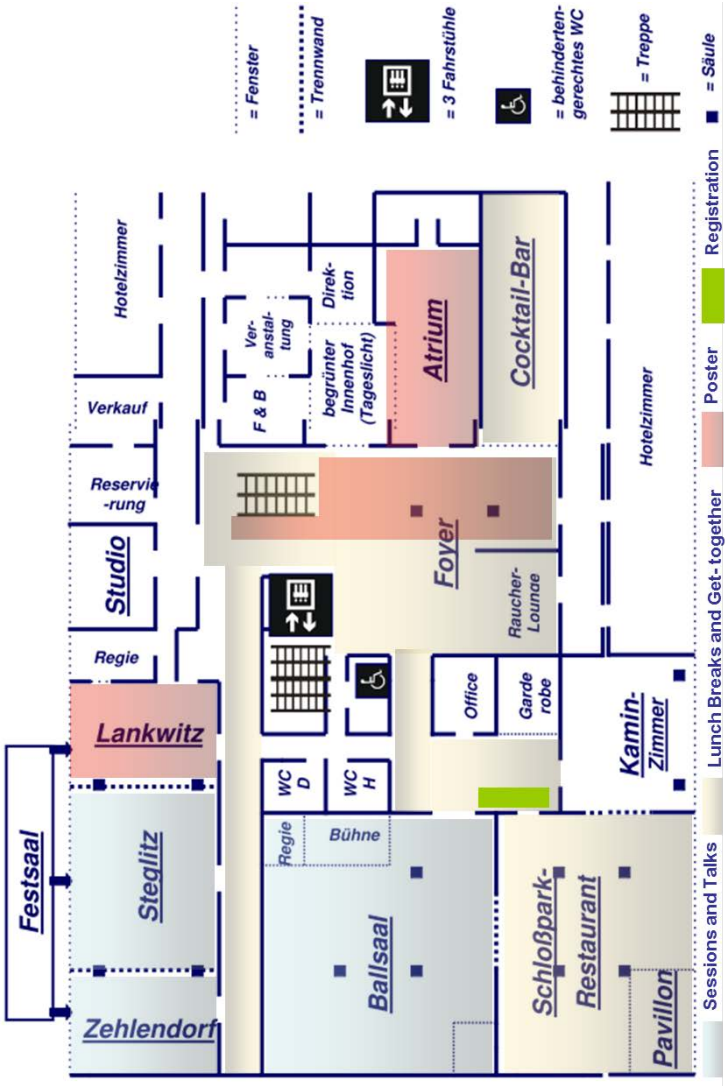
14.30 – 15.15 **One health, food security, and climate changes: What is the connection to zoonotic disease?**
Mo Salman, Fort Collins, USA

15.15 – 16.00 **Deutsches Zentrum für Infektionsforschung (DZIF) – structure and mission of a new multicenter initiative to support translational infection research**
Gerd Sutter, Munich, Germany

16.00 – 16.15 Poster Awards

16.15 Closing Ceremony

Floorplan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Studio
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Reservie-rung
Verkauf
Hotelzimmer
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WC H
Regie
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Ver-anstal-tung
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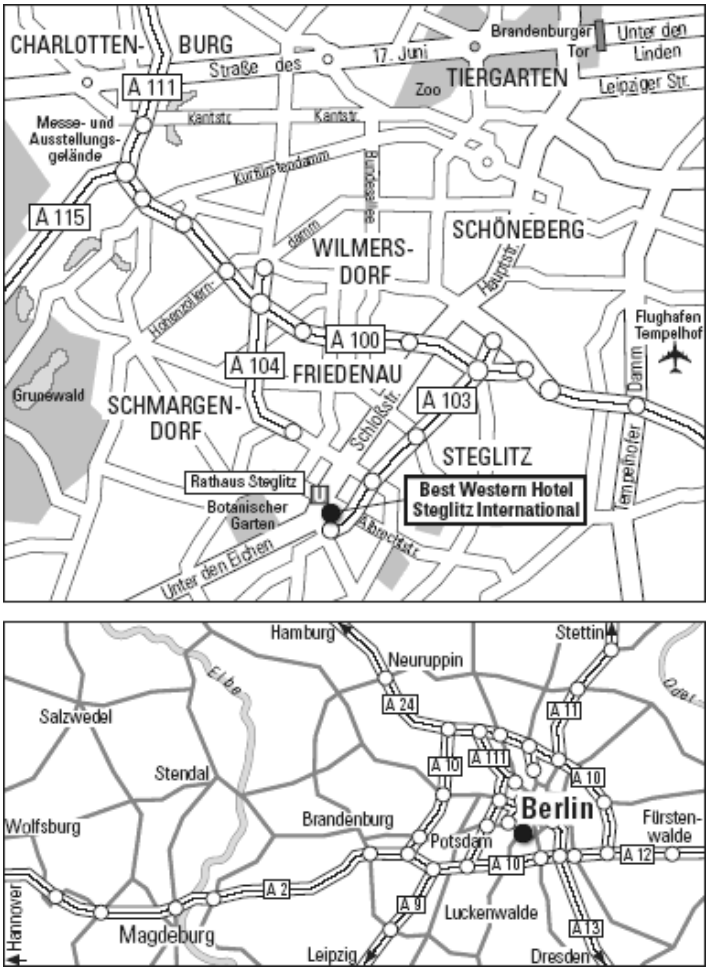
Atrium
Cocktail-Bar
Raucher-Lounge
Office
Garde-robe
Kamin-Zimmer
Hotelzimmer
Schloßpark-Restaurant
Pavillon
Sessions and Talks
Lunch Breaks and Get-together
Poster
Registration

Site Plan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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
Neuruppin
Stettin
Elbe
Oder
Salzwedel
A 24
A 11
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Stendal
Berlin
Wolfsburg
Brandenburg
Potsdam
Fürsten-
walde
A 10
A 12
A 2
A 9
Luckenwalde
A 13
Hannover
Magdeburg
Leipzig
Dresden

General information

Date and venue
11 – 12 October 2012
Best Western Premier Hotel Steglitz International
Albrechtstraße 2, 12165 Berlin
www.si-hotel.com

Scientific committee
Martin Groschup (Greifswald – Insel Riems)
Stephan Ludwig (Münster)
Sebastian C. Semler (Berlin)

Organization
Office of the National Research Platform for Zoonoses
Greifswald-Insel Riems – Münster – Berlin

Review Committee
Members of the internal advisory board of the National Research Platform for Zoonoses in 2011-2012.

Poster Award Committee
The poster awards are selected by the members of the internal and external advisory board of the National Research Platform for Zoonoses.

Plenary Talks
Andrea Ammon, Stockholm, Sweden
Carl Beierkuhnlein, Bayreuth, Germany
Alexander Friedrich, Groningen, Niederlands
Mo Salman, Fort Collins, USA
Gerd Sutter, Munic, Germany
Mary-Kathleen Glynn, Paris, France

Language
The official conference language is English.

Continuous medical education
The National Symposium on Zoonoses Research 2012 is registered for 11 CME points of category A by the Berlin Chamber of Physicians (Ärztekammer Berlin).

Please note, that you need four barcode labels.

Continuous veterinary education
The National Symposium on Zoonoses Research 2012 is registered for 8 hours (ATF-Stunden) by the Federal Chamber of Veterinarians (Bundestierärztekammer).

Oral presentations
Oral presentations should be handed over on a common data carrier at the registration desk on Thursday, 11th October, between 9.00am and 1.00pm. 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 an intermateable file format (PowerPoint or PDF).

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.

About the National Research Platform for Zoonoses

Initiated and funded by the Federal Ministry of Education and Research in Germany, the National Research Platform for Zoonoses started its work in spring 2009 and successfully commences by now the second funding period.

The National Research Platform for Zoonoses develops sustainable and flexible solutions to strengthen research, prevention and therapy of zoonotic infectious diseases.

These objectives will be achieved by the following measures:

- Organization and realization of joint events - also for young researchers at the beginning of their career- which supports 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.
- Initiation and realization of innovative and interdisciplinary pilot projects with cross-sectional character.
- Providing information about zoonotic infectious diseases for the general public.

Our network pursues the wide horizontal cross-linking of human and veterinary medicine and brings together the researchers and the research activities in all fields of zoonoses. To combat zoonotic diseases, interdisciplinary collaboration is necessary. Networking within research consortia gets more and more important. To encounter the challenge of zoonotic infectious diseases, the National Research Platform for Zoonoses supports initiation of new projects and collaborations.

As part of these activities, the National Research Platform for Zoonoses organizes every year the National Symposium on Zoonoses Research with 200-350 participants.

Furthermore, scientific workshops -also for researchers at the beginning of their career- are organised, where specific topics are

presented and discussed. Up to now, five successful workshops were organized in 2012 by the National Research Platform for Zoonoses.
All researchers working on zoonoses in Germany are welcome to join the National Research Platform for Zoonoses. Till now, the National Research Platform for Zoonoses numbers about 500 members.
For further information please visit our website www.zoonosen.net.

Oral presentations

Session Epidemiology, modelling and risk assessment I

11. October 2012
14:00 – 15:30

Room Ballsaal
Chair: Thomas Müller

Prevalence of antibodies against porcine influenza viruses in humans living in areas with a high pig density of Germany.

J. Lange[1], A. Krumbholz[1,2], R. Dürrwald[3], M. Walther[4], T. Müller[5], D. Kühnel[6], P. Wutzler[1], R. Zell[1]

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Keywords: influenza, zoonosis, swine

A previous study conducted in Thuringia revealed a high antibody prevalence against European porcine influenza viruses in persons with occupational contact to pigs. Thuringia is a German federal state with a low pig density. The present serosurvey aimed to analyze the risk of human infection in regions with a high pig density. 622 sera were collected from September 2009 to November 2011, mainly in Westphalia and in the western part of Lower Saxony. Both areas have a very high pig density. The study group included 361 sera from persons with occupational contact to pigs (mainly pig farmers and veterinarians caring for pig herds) and a control group of 261 blood donors without contact to pigs. Sera were analyzed by hemagglutination inhibition (HI) assay against a panel of nine porcine viruses (three strains/subtype swH1N1, swH1N2, and swH3N2) and against human H1N1 and H3N2 viruses including the pandemic H1N1 strain of 2009. Reciprocal HI titers ≥40 were quoted as reactive. Compared to the control group, persons with occupational exposure to pigs exhibited a higher degree of seroreactivity against porcine antigens of the swH1N1 (1.5%; 5.5%) and swH3N2 (11.5%; 18.8%) lineages as well as against the pandemic H1N1 strain of 2009 (13.8%; 23.5%) and against a historic H3N2 strain which is antigentically closely related to currently circulating porcine H3N2 viruses (18.4%; 27.7%). Thus, the zoonotic potential of European porcine influenza viruses was clearly demonstrated.

Prevalence of hepatitis E virus (HEV)-specific antibodies in German domestic pigs – a comprehensive serosurvey

A. Krumbholz[1,2], S. Joel[1], R. Dürrwald[3], R. G. Ulrich[4], P. Dremsek[4], M. Walther[5], P. Wutzler[1], A. Sauerbrei[1], R. Zell[1]

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Keywords: HEV, zoonosis, swine

Currently, an increasing number of autochthonous HEV infections is reported in Germany and other developed countries. Most of these cases are believed to be the result of a zoonotic HEV transmission from pig, wild boar and deer.
Thus, identification and quantification of infectious sources have a high priority. Up to now, there is a lack of profound data concerning the prevalence of HEV-specific antibodies in German domestic pig herds.
This study included 2273 pig sera from fourteen German federal states collected in 2011. Serological testing with the HEV-IgG specific PrioCHECK® ELISA revealed an overall seropositivity of 47.0 % (1069/2273). HEV serostatus differed in dependence of the geographic origin and pigs' age: while 38.6 % of fattening pigs (307/796) presented HEV-specific antibodies, 51.6 % of sows (762/1477) exhibited HEV-specific antibodies.
Another aim of the study was the comparison of different commercially available HEV-assays. 420 randomly selected sera were retested by the HEV Ab-ELISA kit (Axiom), and 64.3 % (270/420) of them were found to be reactive in this assay. However, most of divergent results could not be confirmed by application of a modified commercially available immunoassay (Mikrogen *recom*Line HEV) and were quoted as false-positive.
In conclusion, HEV infection is frequent in the German domestic pig population, and domestic pigs might represent an important reservoir for human HEV infection in Germany.

Bats worldwide carry hepatitis E-related viruses that form a putative novel genus within the family *Hepeviridae*

J. F. Drexler[1], A. Seelen[1], V. M. Corman[1], A. F. Tateno[1], V. Cottontail[2], R. M. Zerbinati[1], F. Gloza-Rausch[1,3], S. M. Klose[1,2], Y. Adu-Sarkodie[4], S. K. Oppong[4], E. K. V. Kalko[2,5], A. Osterman[6], A. Rasche[7], A. Adam[8], M. A. Müller[1], R. G. Ulrich[9], E. M. Leroy[10], A. N. Lukashev[11], C. Drosten[1]

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Keywords: Hepatitis E virus, bats, taxonomy

Hepatitis E virus (HEV) is one of the most common causes of acute hepatitis in tropical and temperate climates. Tropical genotypes 1 and 2 are associated with food- and waterborne transmission. Zoonotic reservoirs (mainly pigs, wild boar and deer) are considered for genotypes 3 and 4 that exist in temperate climates. In view of the association of several zoonotic viruses with bats we analyzed 3,869 bat specimens from 85 different species and five continents for hepevirus RNA. HEVs were detected in African, Central American and European bats, forming a novel phylogenetic clade in the family *Hepeviridae*. Bat hepeviruses were highly diversified and comparable to human HEV in sequence variation. No evidence for transmission of bat hepeviruses to humans was found in over 90,000 human blood donations and individual patient sera. Full genome analysis of one representative virus confirmed formal classification within the *Hepeviridae* family. Sequence- and distance-based taxonomic evaluation suggested that bat hepeviruses constitute a distinct genus within the family *Hepeviridae* and that at least three other genera comprising human, rodent, and avian hepeviruses can be designated. This may imply that hepeviruses invaded mammalian hosts non-recently and underwent speciation according to their host

restrictions. Human HEV-related viruses in farmed and peridomestic animals might represent secondary acquisitions of human viruses, rather than human HEV animal precursors.

Zoonotic potential of hepatitis B virus

A. Blasse[1*], M. Kaiser[2], S. Calvignac-Spencer[1], K. Merkel[1], C. Akoua-Koffi[3], A.V. Adjogoua[3], C. Boesch[4], F. H. Leendertz[1]

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Keywords: HBV, recombination, wild chimpanzees

Recent findings suggest the possibility of cross species transmission of hepatitis B viruses (HBV), which are otherwise known to be restricted to their specific hosts, particularly primates: humans, monkeys or apes. Given that the latter stand for an important component of the bushmeat consumed in sub-Saharan Africa, it is conceivable that zoonotic events might contribute to the emergence of new monkey- or ape-human HBV variants. All the more since the exact reverse case was recently described from one wild born chimpanzee which was shown to carry a chimpanzee-human recombinant strain. To investigate the question of the zoonotic potential of HBV, we characterized HBVs circulating among humans and chimpanzees living in the region of the Taï National Park, Côte d'Ivoire, where bushmeat hunting is frequent. Seven of 69 chimpanzees (based on 91 faecal, 8 liver samples) and 40 of 768 humans were unambiguously infected with HBV.

We obtained full genomic sequences from 15 human and two chimpanzee strains, so as to determine strain phylogenetic relationships and identify possible recombination events. Human and chimpanzee strains do neither appear to be interspersed in phylogenetic reconstructions nor to be recombinant.

Our findings thus seem to indicate that in that region of high risk for zoonotic transmission from apes to humans (as attested by recorded events of, e.g., simian-human transmission of retroviruses), such events are likely to be exceedingly rare when it comes to HBV.

Avian influenza viruses inhibit the major cellular signaling integrator c-Abl

E. R. Hrincius[1], S. Liedmann[1], D. Anhlan[1], T. Wolff[2], S. Ludwig[1] and C. Ehrhardt[1]

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Keywords: influenza A virus, Abl kinase, cellular signal transduction

The non-structural protein 1 (NS1) of influenza A viruses (IAV) encodes several src homology (SH) binding motifs (bm) (one SH2bm, up to two SH3bm), mediating interactions with host cell proteins. Since sequence analysis revealed a different avian (class II SH3bm) versus human (no SH3bm) consensus sequence for the second SH3bm (SH3(II)bm) and based on our former studies, which showed that NS1 binding to CRK adaptor proteins is mediated via this motif, we monitored the regulatory properties of this SH3bm for cellular signaling and the functional consequences in IAV life cycle. We observed that the basal phosphorylation level of CRK was clearly reduced upon infection with different avian IAV harboring an NS1 with an SH3(II)bm in contrast to NS1 of human IAV strains. Reduced activity of the tyrosine kinase c-Abl was identified to be responsible for reduced CRK phosphorylation. Further, direct binding of NS1 to c-Abl was determined. Mutational manipulation of the SH3(II)bm illustrated the necessity of this motif for c-Abl inhibition. Interestingly, Abl kinase inhibition resulted in impaired avian IAV propagation and mutational analysis linked the pronounced inhibition of c-Abl to cytopathic cell alterations upon avian IAV infections. In summary, our data illustrate that NS1 proteins of avian IAV interfere with the kinase activity of c-Abl, a major cellular signaling integrator that controls multiple signaling processes and cell fate regulations apparently including IAV infections.

Immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs) are involved in the antiparasitic defense against *Neospora caninum*

M. Leineweber[1], K. Spekker[1], V. Ince[1], S. Brunder[1], S. K. Schmidt[1], G. Schares[2], W. Däubener[1]

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Keywords: Neospora caninum, antiparasitic defense, GTPases

Mesenchymal stromal cells (MSCs) have a multilineage differentiation potential and provide immunosuppressive and antimicrobial functions. Murine as well as human MSCs are able to restrict the proliferation of T cells. However, species-specific differences in the underlying molecular mechanisms were described. Here we analysed the antiparasitic effector mechanisms active in murine MSCs. We found that murine MSCs, in contrast to human MSCs, are unable to restrict the growth of highly virulent strains of *Toxoplasma gondii* (Type I strain *T. gondii*) after stimulation with IFN-γ. However, the growth of a Type II strain of *T. gondii* was strongly inhibited by IFN-γ activated murine MSCs.

Additional analysis showed that IFN-γ activated mMSCs are also capable of inhibiting the growth of *Neospora caninum*. Detailed co-localization studies indicate that immunity-related GTPases (IRGs) like Irga6, Irgb6 and Irgd were involved in this process. Additional data show that also guanylate binding proteins (GBPs) like mGBP1 and mGBP2 might play a role in the antiparasitic effect of murine MSCs.

These data underline that MSCs, in addition to their regenerative and immunosuppressive activity, might also function as antiparasitic effector cells. However, IRGs are not present in the human genome, indicating a species-specific difference in anti-*T. gondii* effects between human and murine MSCs.

Session Innate and adaptive immune response

11. October 2012
14:00 – 15:30

Room Zehlendorf
Chair: Martin Beer

Type I interferon antagonistic properties of IAV polymerase proteins regulate pathogenicity

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Keywords: influenza A virus, virulence, type I interferon, polymerase proteins

Beside the well-characterized functions in replication and transcription of vRNA, the polymerase complex of influenza A viruses also seems to play a crucial role in the inhibition of the immune response of host cells. In a comparative genome analysis of influenza A virus Puerto Rico/8/34 (PR8) and a PR8 variant with strong interferon stimulating properties we identified eight amino acid differences within the polymerase complex. Mutation of these amino acids led to a dramatically reduced virulence *in vivo*. In addition, our data show clear differences in the induction of the type I interferon (IFN) response in human lung epithelium, but no differences in the accumulation of viral RNA, suggesting an interplay of the viral polymerase complex and virulence regulation on the level of type I IFN inhibition. The interferon antagonistic properties of these virulence determining amino acids could also be verified in the H5N1 virus system. Moreover, analyzing the impact of each single segment we observed striking effects of PB1 and PA proteins on type I IFN inhibition, suggesting an corresponding interplay of the identified amino acids within these two proteins leading to a strong reduction in replication.

In summary, our data revealed interferon antagonistic properties of the polymerase complex, especially PB1 and PA, a function that contribute to virulence of influenza virus.

Early protection of balb/c mice against lethal HPAIV H5N1 challenge infection

S. Röhrs, D. Kalthoff, M. Beer

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Keywords: HPAIV; balb/c mice, immunization, immune response

The primary hosts of highly pathogenic avian influenza (HPAIV) H5N1 viruses are birds, however, this viruses have also the potential to infect mammalians including humans. Therefore, virus-host interactions should be also studied in mammalian hosts, especially concerning the immune response following vaccination or challenge infection.

In our study we used a novel neuraminidase-deleted apathogenic variant of the HPAIV H5N1 strain R65 ("H5N1del") as a vaccine model for a series of immunization/challenge experiments. In a first trial, 6 week old balb/c mice were immunized with a single dose of 104.5 TCID50, and it could be demonstrated that the animals did not show any clinical signs post vaccination and no viral RNA could be detected in organ samples, demonstrating that the neuraminidase-deleted strain was innocuous for mice. In a second trial we intraperitoneally immunized 6 week old balb/c mice 1, 3, 7, 14 and 21 days before a lethal HPAIV H5N1 challenge infection with a single dose of the H5Ndel mutant. Interestingly, the immunization could not completely prevent clinical symptoms, but all animals survived the infection, although only the mice immunized 14 and 21 days before infection had high titers of hemagglutinin-specific antibodies.

In a third trial 6 week old balb/c mice were immunized twice with the inactivated H5Ndel together with an adjuvant. Surprisingly all animals died as fast as the infection control animals although this group had high titers of hemagglutinin-specific antibodies.

In conclusion, a very early onset of immunity against HPAIV seems feasible also in a mammalian infection model using a modified live virus variant, these data should be taken into consideration for future vaccine developments and control strategies.

Critical role of perforin-dependent CD8+ T cell immunity for rapid protective vaccination in a murine model for human smallpox

A. Volz[1]†, M. Kremer[1],†, Y. Suezer[2],†, T. Frenz[4], M. Majzoub[3], K.-M. Hanschmann[2], M. H. Lehmann[1], U. Kalinke[4], G. Sutter[1]

[1]Institut für Infektionsmedizin und Zoonosen, LMU Munich, Germany; [2]Paul-Ehrlich-Insitut, Langen, Germany; [3]Institut für Tierpathologie, LMU München, Germany; [4]TWINCORE, Hannover, Germany.

Keywords: T cells, emergency vaccination, MVA

Although increasing evidence points to cellular immunity playing a critical role in vaccination against viral diseases, vaccine efficacy is mostly associated with induction of antibodies. Here we analyze the immunological mechanism(s) of rapidly protective vaccinia virus (VACV) immunization using mousepox as surrogate model for human smallpox. Surprisingly, we found that fast protection against lethal systemic poxvirus disease solely depended on CD4 and CD8 T cell responses induced by vaccination with highly attenuated modified vaccinia virus Ankara (MVA).

CD4 T cells were critically required to allow for MVA induced CD8 T cell expansion since depletion of CD4 T cells significantly reduced the number of endogenous CD8 T cells specifically recognizing the immunodominant VACV B8R20 27 epitope. Moreover, we observed an essential need for the direct cytotoxic effector function of CD8 T cells as the absence of the cytotoxic protein perforin completely abrogated the protective capacity of immunization. In contrast, selected components of the innate immunity and B cell-mediated responses were fully dispensable for prevention of fatal disease by immunization given 2 days before challenge. In conclusion, our data clearly demonstrate that perforin-dependent CD8 T cell immunity plays a key role in MVA conferred rapid protection against lethal mousepox. Induction of T cell immunity might serve as a new paradigm for treatments that need to fit into a scenario of emergency vaccination.

Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

A. Fischer[1], L. M. Haag[1], B. Otto[1], U. Grundmann[1], A. A. Kühl[2], U. B. Göbel[1], S. Bereswill[1], M. M. Heimesaat[1]

Charité - University Medicine Berlin, [1]Microbiology & Hygiene, [2]Rheumatology and Clinical Immunology, RCIS

Keywords: Campylobacter, enterocolitis, mice

Campylobacter (C.) jejuni is among the leading bacterial agents causing enterocolitis worldwide. Despite the high prevalence of *C. jejuni* infections, intestinal pathogenesis is poorly understood. This is mainly due to the lack of appropriate animal models. In the age of 3 months, adult mice display strong colonization resistance (CR) against *C. jejuni,* Therefore, we investigated CR against *C. jejuni* in 3-weeks-old mice right after weaning and studied intestinal and extra-intestinal immunopathogenesis as well as age dependent differences of the murine colon microbiota.

Within six days following infection, infant mice developed acute enterocolitis as indicated by bloody diarrhea, colonic shortening and increased apoptotic cell numbers in the colon mucosa. Similar to human campylobacteriosis clinical disease manifestations were self-limited and disappeared within two weeks. Strinkingly, *C. jejuni* infection also induced a pronounced influx of immune cells into extra-intestinal sites such as liver, lung and kidney.

These results support the essential role of the microflora composition in CR against *C. jejuni* and demonstrate that infant mouse models resemble *C. jejuni* mediated immunopathogenesis including the characteristic self-limited enterocolitis in human campylobacteriosis. The observed extra-intestinal disease manifestations might help to unravel the mechanisms causing complications such as reactive arthritis or Guillain-Barré-Syndrome.

Campylobacter jejuni induces acute enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via Toll-like-receptor-2 and -4 signaling

S. Bereswill, L.-M. Haag, A. Fischer, B. Otto, R. Plickert, A. A. Kühl, U. B. Göbel, M. M. Heimesaat

Charité - University Medicine (Depts. Micorbiology & Hygiene/ Gastroenterology)

Keywords: Campylobacter, innate immunity, mice

Investigations of the immuno-pathology in human campylobacteriosis are hampered by a lack of suitable vertebrate models. Mice display colonization resistance against the pathogen due to their specific gut microbiota. We have shown that colonization resistance is abrogated in gnotobiotic IL-10 deficient animals which display symptoms of human campylobacteriosis. We generated gnotobiotic IL-10-/- mice by quintuple antibiotic treatment right after weaning. Following oral infection, *C. jejuni* B2 colonized the gastrointestinal tract of gnotobiotic IL-10-/- mice at high levels and induced acute enterocolitis within 7 days as indicated by bloody diarrhea and histopathology of the colon. Intestinal inflammation was characterized by increased numbers of apoptotic cells, T- and B-lymphocytes as well as regulatory T-cells as well as elevated TNF-α, IFN-γ, and MCP-1 concentrations in the inflamed colon. Induction of acute enterocolitis was *C. jejuni* specific, as infection with a commensal *E. coli* strain did not induce disease. Infection of gnotobiotic IL-10-/- mice additionally lacking Toll like receptors (TLR) -4 or -2 revealed that immunopathology is mediated by TLR-4- and, less distinctly, by TLR-2 dependent signalling of *C. jejuni*-LPS and -lipoprotein, respectively.

We present a novel murine *C. jejuni* infection model displaying acute enterocolitis and thus mimicking severe episodes of human campylobacteriosis. This acute model proves useful for further dissecting the immunopathological mechanisms underlying *Campylobacter* infections *in vivo* and to elucidate the interplay between intestinal pathogens, the commensal intestinal microbiota and the innate as well as adaptive immune system of the host.

When innate immunity turns inside out: Novel role of phagocyte extracellular traps against zoonotic bacterial infections?

A. Neumann[1], E. Berends[2], P. Dersch[3], V. Nizet[4], H. Y. Naim[1], M. von Köckritz-Blickwede[1]

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Keywords: phagocyte extracellular traps, Staphylococcus aureus, Yersinia enterocolitica

Phagocyte extracellular traps (PETs) have been recently discovered as a new paradigm in the innate immune function of leukocytes. PETs are released by different immune cells such as neutrophils, macrophages and mast cells after stimulation with mitogens, cytokines, or microbial pathogens. They consist of a nuclear or mitochondrial DNA backbone associated with antimicrobial peptides and histones, which are able to entrap and kill various pathogens. Evidence for a critical role of PETs in innate host defence has come from recent experimentation manipulating the microbial side of the host–pathogen interactions. For example, we could show that methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved a mechanism to avoid PET-based immune clearance through PET degradation by nucleases and thereby contributes to virulence in a mouse model of pneumonia. However, so far little is known about the role of PETs during food-borne zoonotic bacterial infections that cause gastro-intestinal disorders. The goal of current work is to analyze the role of PETs during infections with *Yersinia enterocolitica*. In a first attempt, it could be shown that different serotypes of *Y. enterocolitica* express nuclease activity during its midlog growth phase. Current experiments are analyzing the ability of different *Y. enterocolitica* strains to avoid entrapment and killing by PETs. This project will provide novel insight into the role of PETs in host defence against zoonotic bacterial infections.

Session Pathogen-cell interaction I

11. October 2012
14:00 – 15:30

Room Steglitz
Chair: Christian Menge

Study of host-pathogen interactions in *Chlamydia psittaci*- and *Chlamydia abortus*-infected chicken embryos reveals marked differences in dissemination and regulation

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Keywords: Chlamydia infection; embryonated chicken eggs; host immune response

The mechanisms and factors determining pathogenicity and host adaptation of the various *Chlamydia* species are still largely unknown. In the present study, we compared the avian pathogen *C. psittaci* and the ovine pathogen *C. abortus*, both of them zoonotic agents, with regard to their virulence properties and capability to elicit immune defence reactions in an embryonated chicken egg model.
As shown by immunohistochemistry and quantitative real-time PCR, *C. psittaci* displayed a significantly better capability of disseminating in the chorioallantoic membrane (CAM) and internal organs than *C. abortus*. The higher infectious potential of *C. psittaci* in birds was underlined by significantly higher mRNA expression rates of essential chlamydial genes, such as *incA*, *groEL* (in CAM, liver, spleen), *cpaf* and *ftsW* (in CAM). Although the immune response to both pathogens was similar, *C. psittaci* elicited higher macrophage numbers and generally stronger expression of a sub-set of immune-related proteins (iNOS, IL-1β, IL-8, LITAF).
The data imply that invasiveness of *Chlamydia* spp. and propagation in the host are not solely dependent on the level of host immune response, but also the expression of bacterial virulence factors. The fact that *C. psittaci* has coped far better than *C. abortus* with the avian embryo's response by upregulating its virulence-associated genes may be a key to understanding the mechanisms underlying host adaptation and etiopathology.

Characterization of the potential virulence factors CAB063 and CAB821 of *Chlamydia abortus*

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Keywords: Chlamydia abortus, immunoreactive proteins, virulence factor

The obligate intracellular bacterium *C. abortus* is the causative agent of enzootic abortion of ewes and can also be transmitted to humans. Due to the lack of genetic tools for *Chlamydia* our understanding of pathogenesis has to be improved by alternative approaches. Proteomic profiling may provide information about pathogenic mechanisms as the humoral host response can lead to the identification of bacterial antigens involved in bacteria-host cell-interaction. We screened *C. abortus* proteins by serological 2D proteomic analysis and by screening of a *C. abortus* specific expression gene bank in *E. coli* using sera from sheep and human sera. We identified about 50 chlamydial antigens, among them the "hypothetical" proteins CAB063 and CAB821 predicted to be secreted by the Type III secretion system into the host cell cytoplasm. We characterized both putative virulence factors by gene expression analysis at RNA (qPCR) and protein level throughout the developmental cycle and analyzed their subcellular localization by immunofluorescence microscopy and cell fractionation using polyclonal antibodies generated in mice and rabbits. Transfection experiments in order to confirm preliminary results showing CAB063 to be localized within the host cell nucleus are under way. Subcellular localization of CAB821 showed its enrichment at opposing membranes of multiple chlamydial inclusions indicating a role of this protein in the fusion process.

Modulation of host gene expression upon antimicrobial treatment of persistent *Chlamydia psittaci*

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Keywords: Chlamydia psittaci, persistence, antimicrobial treatment

Chlamydiae are intracellular pathogens that are able to persist in the host cell in a viable but uncultivable state. This persistence is related to chronic disease in that antimicrobial therapy may fail.
An IFNγ-mediated persistence model for *C. psittaci* was established in A549 cells. Different antimicrobial treatment strategies using macrolides, doxycycline, and quinolones and combinations with rifampicin resulted in an incomplete eradication of persistent *C. psittaci* characterized by reisolation of viable bacteria and only partial down-regulation of chlamydial gene expression. Additionally the response of epithelial cells to chlamydial infection was investigated by microarray studies and the results were confirmed by real-time RT-PCR. *IL-8* and *TNFRSF9*, which were 5-fold up-regulated upon infection, and *ADAM15* which was 2-fold down-regulated were selected as marker of the host response for further experiments. Antibiotic treatment led to altered expression of *IL-8* and *TNFRSF9* in active and persistent infection. *IL-8* was 7-fold and *TNFRSF9* 3-fold down-regulated following antimicrobial treatment of persistent infection.
The results of the study demonstrate that the host cell response to active and persistent chlamydial infection is differentially modulated upon antimicrobial treatment. These findings may help to better understand inflammatory processes during chronic *C. psittaci* infection and to evaluate the efficacy of an antimicrobial therapy by analysing representative inflammation-related host cell genes.

Comparative analysis of the six botulinum neurotoxin A subtypes

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Keywords: Subtypes of botulinum neurotoxin A, binding, biological activity

Botulinum neurotoxins (BoNTs) are the most poisonous bacterial protein toxins causing the zoonosis botulism due to blockade of acetylcholine release at the neuromuscular junction. The seven serologically different BoNT types A to G are composed of four domains which play individual roles in the intoxication mechanism. The C-terminal H_C fragment of BoNT/A contains the two receptor binding sites for gangliosides and the synaptic vesicle glycoproteins SV2A, SV2B and SV2C. The N-terminal light chain (LC) is a Zn-metalloproteases, which specifically hydrolyses the neuronal SNARE protein SNAP-25 that is part of the vesicle exocytosis machinery.
Systematic genome sequencing of isolated *C. botulinum* strains encoding BoNT/A revealed up to 17 % divergence of the BoNT amino acid sequence. These BoNT/A variants were grouped into currently six BoNT/A subtypes (A1-6). Such sequence differences can already affect i.a. the biological activity, immunological detection and neutralisation of BoNT by therapeutic antibodies and hence necessitates the identification of all subtypes and characterisation of their biochemical properties.
Here, we analysed the five published BoNT/A1-A5 and a novel subtype (BoNT/A1-6). We compared the binding of their isolated H_C fragment to their receptors SV2A, SV2B, SV2C and to synaptosomes. Furthermore, we tested the catalytic activity of the isolated LCs. Their overall biological activity was tested using the mouse *phrenic nerve* hemidiaphragm (MPN) assay.

Expression of host cell cycle regulators during infections of skeletal muscle cells with *Toxoplasma gondii*

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Keywords: Toxoplasma gondii, Skeletal Muscle Cell, T. gondii-host cell interaction

Toxoplasma gondii is an apicomplexan parasite the transmission of which to humans occurs regularly via the intake of undercooked meat products containing bradyzoites, the persistent stage of *Toxoplasma*.
Our previous results revealed restricted parasitic growth in differentiated SkMCs compared to proliferating SkMCs and fibroblasts *in vitro*. This developmental inhibition is accompanied by higher bradyzoite formation in differentiated SkMCs as detected by staining the cyst wall using specific antibodies CC2 or *Dolichos biflorans* lectin and BAG1 quantification.
Because CDA1 is a regulator of bradyzoite formation in human fibroblasts, we analysed its expression in SkMCs. We show an increasing expression of CDA1 and its downstream target gene p21 during myogenic differentiation. Moreover, CDA1 but not p21 expression further increased in myotubes after infection. Currently we are investigating the impact of CDA1 on *Toxoplasma* development in myotubes using RNAi.
For identification of cell-type specific interactions between *Toxoplasma* and its host cell, high-throughput RNA sequencing (Illumina) of SkMCs, neurons, astrocytes and fibroblasts after infection was carried out. Interestingly, many cell cycles regulators and genes responsible for nucleotide binding were upregulated in SkMCs but not in fibroblasts after infection suggesting a putative role in *Toxoplasma* persistence in differentiated SkMCs. The analysis of neuron and astrocyte transcriptomes is currently in progress.
In conclusion, the state of differentiation of SkMCs impacts *T. gondii* replication and stage differentiation *in vitro*. In addition the host cell cycle regulation seems meaningful for *Toxoplasma* persistence in differentiated SkMCs.

Coxiella burnetii harbors several anti-apoptotic type IV secretion system substrates

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Keywords: Coxiella burnetii, apoptosis, type IV secretion system

Manipulation of host cell apoptosis is a virulence property shared by many intracellular pathogens to ensure productive replication. For the obligate intracellular pathogen *Coxiella burnetii* anti-apoptotic activity, which is depending on a functional type IV secretion system (T4SS), has been demonstrated. Additionally, the *C. burnetii* T4SS-effector protein AnkG was identified to inhibit pathogen-induced apoptosis, possibly by binding to the host cell mitochondrial protein p32 (gC1qR). However, it was unknown whether AnkG alone is sufficient for apoptosis inhibition or if additional effector proteins are required. Here, we identified two T4SS-effector proteins CaeA and CaeB (***C****oxiella burnetii* **a**nti-apoptotic **e**ffector) that inhibit the intrinsic apoptotic pathway. Further characterization showed that CaeB blocks apoptosis very efficiently, while the anti-apoptotic activity of CaeA is weaker. We demonstrated that CaeB did not cause alteration in the protein level of apoptosis regulators, but rather acted downstream of Bax and upstream of caspase 3. Additionally, our data indicated that CaeB inhibits apoptosis at the mitochondrial level, but did not bind to p32. Taken together, our results demonstrate that *C. burnetii* harbors several anti-apoptotic effector proteins and suggests that these effector proteins use different mechanism(s) to inhibit apoptosis.

Session Novel methods and diagnostics

12. October 2012
09:30 – 10:30

Room Zehlendorf
Chair: Albrecht von Brunn

Functional characterisation of seven botulinum neurotoxin type B subtypes

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Keywords: Subtype, Botulinum neurotoxin B, biological activity

Botulinum neurotoxins (BoNTs) are extremely potent bacterial protein toxins causing the disease botulism due to blockade of acetylcholine release at the neuromuscular junction. The seven serologically different BoNT types A to G are composed of four domains which play individual roles in the intoxication process. The C-terminal H_C fragment of BoNT/B contains the two receptor binding sites for gangliosides and the synaptic vesicle proteins synaptotagmin (Syt)-I and -II. The N-terminal light chain (LC) constitutes the catalytically active domain which specifically hydrolyses synaptobrevin 2 in the neuronal cytosol.

Systematic genome sequencing revealed 8 BoNT/B variants called subtypes exhibiting up to 8 % amino acid sequence divergence which can already affect i.a. their biological activity, immunological detection and neutralization by therapeutic antibodies.

Therefore it is important to identify all BoNT subtypes and characterise their biochemical properties.

Here, we analysed the seven subtypes of BoNT/B (BoNT/B1-B5 and B7+B8). We compared the binding of their isolated H_C fragments to Syt-I and Syt-II as well as to synaptosomes. Furthermore, we verified the catalytic activity of the isolated LCs. Their biological activity was tested using the mouse *phrenic nerve* hemidiaphragm (MPN) assay.

In conclusion, less than three amino acid exchanges in BoNT/B2, B3 and B8 significantly modulated the affinity to Syt-I and Syt-II and their catalytic activity, respectively.

Cell culture contaminations – still relevant in the age of technology?

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Keywords: Simian Virus 5, cell culture contamination

Products generated from cell culture are for many experiments the basis for all further studies to come: virological and immunological studies, proteomics, gene expression profiling, transcriptome analysis, and many more. In our working group infectious studies in animals were performed. The utilised virus was propagated in cell culture – previously screened as mycoplasma negative – and purified by ultracentrifugation. Virus-containing pellets were resuspended in PBS. Parallel in a different working group a Paramyxovirus (PMV) contamination was visualized by electron microscopy while performing virus isolation. It was characterized via random PCR and sequence analysis as Simian Virus 5 (SV-5). A general testing for SV-5 revealed that the cells used for virus propagation in the animal studies were also highly contaminated with SV-5. The same was true for the virus stocks after ultracentrifugation. SV-5 is supposed to be a common cell culture contamination (along with other PMV). It replicates in high copy numbers but shows no CPE and is therefore not recognised without specific testing. As an implication of former times, cell culture contaminations were described repeatedly – but in the age of "–omics" cell culture contaminations of various viruses are more relevant than ever.

Validation and establishment of an optimized DNA-microarray system for the non targeted detection of viruses

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DNA microarrays are highly parallel biosensors which allow a fast, standardized, simultaneous detection and identification of many different viruses in one or more samples. In this study we used an 8x15k Pan Viral Chip developed within the European Network of Excellence project "EPIZONE" and printed by Agilent. These arrays contain catcher oligonucleotides specifically detecting 1960 different virus families/species.

The array was validated and its sensitivity was determined using two different viruses. On the one hand, we used the classical swine fever virus (CSFV) strain "Kozlov", a positive stranded RNA virus. On the other hand, a double stranded DNA virus (modified Vaccinia Ankara; MVA) was used. The nucleic acid samples (DNA or RNA) were processed prior to hybridization onto the array using three different workflows in comparison.

In order to distinguish between positive and negative signals in the raw data, data were analysed with three different methods in comparison. First, data were processed using the "DetectiV" R-package, initially developed for analysis of the EPIZONE chip data. Second, the well-established "Limma" R-package was used for data analysis. Third, Z-score transformation in combination with calculation of the accompanying P-values was done. The results of the different data analysis methods were compared.

With the established optimal combination of sample processing and subsequent data evaluation, a series of different RNA and DNA viruses, including the novel Schmallenberg virus, and numerous sample materials were analysed. It could be shown that the established workflow can successfully be used for the very broad virus detection in cell culture and diagnostic samples.

Rapid analysis of large diagnostic metagenome datasets – the Schmallenberg virus example

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Keywords: Next Generation Sequencing, Metagenomics, Diagnostics

Next generation sequencing (NGS) enables the comprehensive sequencing of genomic material from diverse samples, regardless of source and composition. The output of the different NGS technologies differs in the mean length and quantity of the obtained sequence fragments (reads). Irrespective of the sequencing technology, the metagenomic datasets consist of some tens of thousands up to millions of single sequence reads. These large datasets pose a challenge for the diagnostic metagenomic approach because the decisive information may come from only a single read that must be identified amongst the information overload of non-pathogen related reads like host derived sequences.
Therefore, we developed an intelligent, automated workflow for reliable classification of every single read into the different taxa. For an optimized performance, i.e. for the fast and accurate processing of the huge datasets, we rely on a combination of different alignment algorithms such as assemblies and mappings implemented in the Genome Sequencer software and NCBI BLAST tools. First, the data quantity is reduced based on organisms detected within consensus sequences assembled from data subsets. Secondly, different BLAST variants assign remaining sequences. Finally, all detected organisms are listed in the result protocol grouped according to the taxonomy. The complete analysis of a dataset of approximately 100.000 reads usually requires only 30 to 120 minutes instead of days up to weeks.
The presented software pipeline which proved its power in the detection of the Schmallenberg virus (SBV) late in 2011 can universally be applied to the analysis of all metagenomic data including those with suspected zoonotic agents. The initial hint for the identification of SBV as causative agent of an undiagnosed disease last summer and fall was the discovery of seven orthobunyavirus reads with approximately 69% homology to Akabane virus amongst approximately 27.000 reads in the metagenomic data generated from cattle samples.

Session Antimicrobial use and resistance

12. October 2012
09:30 – 10:30

Room Ballsaal
Chair: Lothar H. Wieler

Livestock-associated MRSA of the clonal complex 398: Characterization of infection properties

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Keywords: MRSA CC398, virulence potential, adhesive properties

Recently, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of the emerging clonal complex (CC) 398 were recognized as a challenge in healthcare facilities.
Yet, the pathogenic mechanisms of endemic LA-MRSA with regard to species barrier transmission, colonization and disease formation are largely unknown. To address these processes, the adhesive abilities and the virulence potential of epidemiologically relevant zoonotic and non-zoonotic MRSA were analyzed. We studied the adherence to human endothelial and epithelial cells and cell matrix components, analyzed the cytotoxic properties of staphylococcal culture filtrates and investigated virulence determinants like the *S. aureus* alpha-toxin (encoded by *hla*) at the level of gene expression.
First results showed clear differences between LA-MRSA and prevalent hospital acquired- (HA) and community acquired- (CA) MRSA lineages regarding their adhesion. LA-MRSA CC398 isolates obtained from animal sources possessed reduced adhesion capacities to fibronectin compared with CC398 isolates from human origin as well as with HA- and CA-MRSA. Strain-specific variations in adhesion capacities identified by an ELISA-based adhesion assay correlated with adhesion force measurements determined by atomic force microscopy (AFM). Regarding the cytotoxicity, MRSA CC398 isolates showed higher cytotoxic effects on epithelial cells compared to CA- and HA-MRSA isolates in a cell culture assay.

VIM-1 carbapenemase carrying *Escherichia coli* and *Salmonella enterica* from livestock farms.

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Keywords: carbapenemase, VIM-1, livestock

Carbapenems are critically important antimicrobials. Carbapenem non-susceptible Enterobacteria seemed to be mainly restricted to hospitals and the community. However, as presented here, they are also present in livestock farms.
Third generation cephalosporin resistant *E. coli* and *Salmonella* collected in German livestock farms within a national ESBL-surveillance project (www.reset-verbund.de) were tested for their susceptibility to several antimicrobials and presence of ESBL/ AmpC/carbapenemases encoding genes (PCR/sequencing).
Carbapenemase-producing isolates were analysed by MLST, XbaI-PFGE; plasmids by S1-PFGE, replicon PCR-typing, mating and transformation experiments. Two *E. coli* and three *Salmonella enterica* were positive for the bla_{ACC-1} AmpC-gene and the bla_{VIM-1} carbapenemase-gene (both located on HI12 plasmids). The bla_{VIM-1} gene was located in a class 1 integron with *accC4* and *aadA1,* and *sul1* (amikacin-kanamycin/ streptomycin-spectinomycin/ sulfonamides resistance). The plasmids also carried *strA/B* (streptomycin resistance), and in *Salmonella*, *catA* (chloramphenicol resistance) and a non-characterized trimethoprim resistance gene as well. These isolates are the first carbapenemase producing *E. coli* and *Salmonella* desribed in livestock so far. The epidemiological importance of carbapemenase-producers in livestock could be underestimated and is a Public Health concern that deserves further surveillance.

Profiling of antibiotic resistance genes in reservoir bacteria isolated from different sources

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Keywords: Enterococcus faecalis, Escherichia coli, gene profiles

Cross transfer of antimicrobial resistance from animals to humans is frequently discussed even if little is known about the actually occurring horizontal spread. Therefore, a representative number of *E. coli* (n = 289) and *E. faecalis* (n=424), isolated from diseased and healthy humans and pigs, was examined for the presence of selected resistance genes (n=23). Significant differences were observed between the genetic profiles of the reservoir bacteria from humans and pigs, indicative of a provenance based transfer limit. Briefly, the most common *tet*-gene in *E. coli* was *tet*(A) in porcine, but *tet*(B) in human isolates. Streptomycin resistance was mostly mediated by *str*(A)/*str*(B) in porcine, but by *str*(A)/*str*(B)/*aad*(A) in human strains. The prevalence of *tet*(L), *tet*(S), *erm*(A) and *erm*(B) was higher in porcine *E. faecalis*. Gene profiles were most similar in healthy and diseased pigs (82 %) and most dissimilar in healthy humans and diseased pigs (61 %). Diseased pigs had the highest percentage of unique profiles (11,3 %). Porcine *E. coli* carried more often 1 - 4 of the investigated genes; co-occurrence of 5 or 6 genes was more present in human *E. coli*. By contrast, *E. faecalis* of human origin carried more often 1 or 2 resistance genes, whereas porcine strains carried more often 3 - 5 genes. The huge diversities between the genetic profiles indicate that a cross transfer between mircroorganisms from different sources may be less common than within populations of the same source.

Replication of human coronaviruses SARS-CoV, HCoV-NL63 and HCoV-229E is inhibited by the drug FK506

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Keywords: coronavirus inhibition, FK506-binding proteins, tacrolimus

FK506-binding proteins represent a subset of the immunophilin protein family including the structurally unrelated cyclophilins. They are expressed at high levels in almost all tissues, and they are characterized by the presence of at least one peptidyl-prolyl isomerase domain (PPIase) excerting regulatory or chaperone functions. FK506 (tacrolimus) is a bacterial macrolide that binds to FKBPs. It excerts two independent functions: a) The FK506-FKBP complex binds to the cellular phosphatase Calcineurin (as a kind of freak of nature) thus inactivating the important immunologic Calcineurin/NFAT (Nuclear Factor of Activated T-cells) pathway. The drug can therefore be used as an immunosuppressant to prevent organ rejection in transplant patients. B) independently, FK506 inhibits PPIase and chaperone activities of FKBPs resulting in misfolding and inactivity of cellular and also certain viral proteins in virus-infected cells.

We have recently shown the dependence of Coronavirus (CoV) replication on active immunophilin/cyclophilin pathways (Pfefferle et al., 2011, PLoS Pathog 7(10): e1002331). Here we report that the drug FK506 (Tacrolimus) inhibits strongly the growth of human coronaviruses SARS-CoV, HCoV-NL63 and HCoV-229E at low, non-cytotoxic concentrations in cell culture (Carbajo et al., 2012, VirRes 165, 112–117). As shown by plaque titration, qPCR, Luciferase- and green fluorescent (GFP) reporter gene expression, replication is diminished by several orders of magnitude. Knockdown of the cellular FK506-binding proteins FKBP1A (=FKBP12) and FKBP1B (=FKBP1.6) in CaCo2 cells prevents replication of HCoV-NL63, suggesting the requirement of these members of the immunophilin family for virus growth.

Session Pathogen-cell interaction II

12. October 2012
09:30 – 10:30

Room Steglitz
Chair: Ralph Goethe

Correlation between *Mycobacterium* subsp. *paratuberculosis* infection and colitis

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Keywords: Mycobacterium paratuberculosis, mouse model, Inflammatory Bowel Disease

Mycobacterium avium subsp. paratuberculosis (MAP) causes Johne's disease (JD), which is a chronic inflammatory bowel disease (IBD) in ruminants. An association between MAP infection and Crohn's disease (CD), a chronic IBD in human has been found. Similarity of pathology of JD and CD in clinical symptoms like weight loss and diarrhea suggested a common cause. However, MAP being the cause of CD is still under debate. In this direction, a detailed study has been limited by the lack of a suitable mouse model for MAP infection. The hypothesis was derived that MAP might not be causing but exacerbating the already existing IBD.

Here, in our studies we show that colitis was worsened when DSS-treated mice were infected with MAP. The exacerbation of the infection was evident from the increasing liver and spleen weight over the time after infection. Enlargement and increasing of granuloma in liver also was observed in DSS-MAP mice. Furthermore, viable bacteria in intestine were found only in DSS-MAP infected mice at three weeks after infection. Interestingly, after secondary infection with MAP, only DSS-MAP mice showed the colon length shortening as well as diarrhea, which both are the clinical symptoms of IBD. Other mycobacterium species, *M. avium* and *M. hominisuis* failed to induce similar effects. Furthermore, Rag2 deficient mice lacking B and T cells were not effected to the same degree as normal wild type mice. Initial experiment showed an increase in the number of B and T cells in the mesenteric lymph nodes after secondary infection. Taken together, these results are consistent with the previous hypothesis that MAP exacerbates the existing colitis and the adaptive immune system contributes to this effect.

Establishment of molecular methods to detect autochthonous flea species on small mammals and investigation of their associated pathogens

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Keywords: fleas, rodents, zoonotic pathogens

Small mammals such as rodents carry several different ectoparasites including fleas. Rodents and ectoparasites may harbour and transmit zoonotic bacteria and eventually viruses. Recently, we found wild rodents positive for *Rickettsia felis*, a usually cat-flea-transmitted pathogen. In order to study maintenance of pathogens between possible reservoirs and/or vectors we investigated fleas collected from wild rodents and their associated pathogens. 487 small mammals (193 *Apodemus* sp., 278 *Myodes glareolus,* 12 *Microts agrestis*, 4 *Sorex araneus*) from the National Park Bohemian Forest collected in 2010 were investigated for fleas, ticks and lice and infestation rates were determined. On 60 fleas collected in 2008 from rodents of the same area classical morphological species determination was performed. In order to classify flea species by molecular methods, PCR based on the ribosomal RNA and mitochondrial cytochrome B were established. Of all samples, 200 flea-specific sequences were generated; four of these fleas were also determined morphologically. Two genotypes of *Ctenophtalmus agyrtes, Peromyscopsylla sylvatica, Malareus peniciliger, Hystrichopsylla talpae* and a *Ceratophyllus*-like species could be determined by cytochrome B sequencing. Interestingly, only a low prevalence (2%, n=5/240) of rickettsial DNA was detected in fleas so far. Further investigations will be performed to determine more rodent-associated flea species and in particular their associated zoonotic pathogens.

Infectivity in peripheral muscle but not in the lymphoreticular system of cattle clinically affected challenged with atypical BSE

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Keywords: BSE, atypical, pathogenesis

Since 2004, two different forms of so-called atypical BSE (L-type and H-type) have been diagnosed in many European countries as well as in North America and Japan in low numbers in animals older than eight years. These atypical forms differ from classical BSE by the clinical picture and by the biochemical properties of the accumulated pathological prion protein (PrP^{Sc}). From the epidemiological data available so far, a spontaneous origin has been postulated for both forms. After a higher zoonotic potential of L-type atypical BSE has been demonstrated by transmission studies in transgenic mice and in non-human primates, we decided to analyse the agent distribution in the periphery of L-type BSE infected cattle. We have therefore performed a mouse bioassay in highly sensitive bovine PrP transgenic mice (Tgbov XV) using peripheral tissues of the muscular, neuronal and lymphoreticular system of cattle that were experimentally challenged with L-type and H-type BSE by the intracranial route. It could be confirmed that, like in classical BSE, the lymphoreticular system plays no role in the agent distribution. In contrast, the truncus vagus ventralis and the *M. semitendinosus* of clinically affected cattle were shown to contain low levels of infectivity, albeit the PrP^{Sc} concentrations were below the detection limit.
We can therefore conclude that the pathogenesis of atypical BSE basically follows the same pattern that is known for classical BSE. In earlier studies with classical BSE, we revealed a centrifugal spread of infectivity and PrP^{Sc} from the central nervous system to the periphery at the late stage of the disease, which we conclude also to be true for atypical BSE.

Ebola virus entry into host cells: Analysis of new receptor candidates

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Keywords: Ebola, TIM-1, NPC-1

Ebola viruses (EBOV) cause hemorrhagic fever in humans with high case-fatality rates. The viral glycoprotein (GP) mediates infectious viral entry into host cells. However, the host cell receptors engaged by GP for cellular entry are poorly defined. Recently, the phosphatidylserine receptor TIM-1 and the endosomal cholesterol transporter NPC-1 were reported as EBOV receptors. In addition, a role for the Tyro-3 kinase Axl has been suggested. Here, we investigated if these receptors contribute to EBOV entry into cell lines and macrophages, major viral target cells.

When analyzing cell lines, we found that directed expression of TIM-1 can increase EBOV-GP-mediated entry, while knock-down of endogenous TIM-1 or Axl can inhibit entry. However, expression of both receptors did not correlate with susceptibility of cell lines to EBOV-GP-driven infection. In contrast, NPC-1 inhibitors reduced EBOV-GP-mediated entry into all cell lines tested, suggesting that NPC-1 is universally required for EBOV entry. Human macrophages did not express appreciable amounts of TIM-1 and Axl mRNA and siRNA knock-down of these receptors had no impact on GP-driven entry. In contrast, blockade of NPC-1 and integrin α5 inhibited infection of macrophages and viral uptake into these cells was dependent on macropinocytosis. Our results identify factors important for EBOV infection of macrophages but also suggest that the receptor(s) responsible for viral binding to these important target cells is still elusive.

Session Pathogen-cell interaction III

12. October 2012
11:30 – 13:00

Room Zehlendorf
Chair: Christian Drosten

Acquisition of new protein domains by SARS-coronavirus: Functional and structural dissection of the SARS-unique domain (SUD)

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Keywords: macrodomain fold, G-quadruplex, poly(A)-binding protein-interacting protein 1

The largest non-structural protein (Nsp) within the coronaviral polyprotein is Nsp3 (1922 amino-acid residues in the case of SARS-CoV). In the SARS-virus, Nsp3 includes the SARS-unique domain (SUD), which is only found in this coronavirus and related betacoronaviruses from certain bats, but not in any other CoV including other betacoronaviruses. Therefore, it has been speculated that the SUD may be involved in the extreme pathogenicity of SARS-CoV.

In an extended study of the SUD, we revealed the following through X-ray crystallography, site-directed mutagenesis, reverse genetics, and "interactomics":

1. The domain consists of three subdomains, N, M, and C.
2. SUD-N and SUD-M exhibit a macrodomain fold similar to the preceding SARS-CoV X domain, but they fail to bind ADP-ribose.
3. Instead, the SUD and its subdomains N and M specifically bind to oligoribonucleotides forming G-quadruplexes.
4. Mutation of selected lysine residues on the surface of SUD subdomains N and M abolishes G-quadruplex binding.
5. SUD-N (as well as the X-domain) are not essential for replication, but SUD-M and SUD-C are.
6. SUD-N specifically interacts with the middle domain of human poly(A)-binding protein interacting protein 1 (Paip-1M).
7. The 3D structure of Paip-1M is built from 5 HEAT repeats.
8. The complex between SUD-N and Paip-1M comprises a relatively small interface, perhaps leaving space for another interaction partner in the translation initiation machinery.

The SARS-Coronavirus-host interactome: Identification of cyclophilins as targets for pan-Coronavirus inhibitors

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Keywords: virus-host interactome, cyclosporine A, CoV inhibition

Coronaviruses (CoV) are important human and animal pathogens that induce fatal respiratory, gastrointestinal and neurological disease. The outbreak of the severe acute respiratory syndrome (SARS) in 2002/2003 has demonstrated human vulnerability to CoV epidemics. Neither vaccines nor therapeutics are available against human and zoonotic CoV. Knowledge of host cell proteins that take part in pivotal virus-host interactions could define novel broad-spectrum antiviral targets. Using a systems-biology approach, we identified in this study by genome-wide yeast-two hybrid interaction screening cyclophilins as interaction partners of the CoV non-structural protein 1 (Nsp1, (Pfefferle et al., 2011, PLoS Pathog 7: e1002331). Interactions of Nsp1 with cylophilins modulated the Calcineurin/NFAT pathway and led to altered cytokine secretion, a key feature of viral immunopathogenesis. Conversely, inhibition of cyclophilins, integral components of the NFAT pathway when complexed with Cyclosporine A (CspA), inhibited replication of CoV of all genera, including SARS-CoV, human CoV-229E and -NL-63, feline CoV, porcine transmissible gastroenteritis virus [TGEV] as well as avian infectious bronchitis virus. Non-immunosuppressive derivates of CspA could serve as broad-range coronaviral inhibitors against emerging new CoV and ubiquitous pathogens of humans and livestock as well.

The influenza virus and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in the human respiratory and gastrointestinal tracts

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Keywords: influenza A virus, SARS-CoV, type II transmembrane serine proteases

Influenza viruses and SARS-coronavirus (SARS-CoV) depend on activation by host cell proteases for acquisition of infectivity. TMPRSS2 and HAT, members of the type II transmembrane serine protease (TTSP) family, activate influenza A viruses (FLUAV) and SARS-CoV in cell culture. However, it is at present largely unclear to which extent these proteases are expressed in viral target cells in human tissues.

Immunohistochemistry revealed that TMPRSS2 and HAT are co-expressed with 2,6-linked sialic acid, the major receptor determinant of human FLUAV, throughout the respiratory tract. In addition, ACE2, the cellular receptor employed by SARS-CoV for host cell entry, and TMPRSS2 were co-detected on several sites of the upper and lower aerodigestive tract, including type II pneumocytes, important viral target cells. Furthermore, TMPRSS2 of avian and porcine origin activated the FLUAV hemagglutinin, indicating that this protease might support FLUAV spread in reservoir, intermediate and human hosts. Finally, we could show that TMPRSS2 from mice, which are frequently used as animal model for FLUAV infection, activates FLUAV, indicating that mice can be used to study the role of TMPRSS2 in viral spread and pathogenesis. In summary, our results demonstrate that TMPRSS2 and HAT are co-expressed in important FLUAV and SARS-CoV target cells and could therefore support viral spread in the human host.

A reverse genetics approach to study the determinants for dsRNA-binding and PKR inhibition of the influenza A virus NS1 protein

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Keywords: NS1, PKR activation, RNA-binding

The non-structural protein 1 (NS1) of influenza viruses functions in inhibiting the antiviral state in infected cells. While NS1 proteins of influenza A and B viruses (A/NS1 and B/NS1, respectively) have only 20% sequence identity, they have a conserved RNA-binding domain and share functions as inhibition of the antiviral kinase PKR. We have previously shown that distinct basic amino acid residues of the B/NS1 protein are required for dsRNA-binding and silencing of PKR activation. In contrast, the A/NS1 protein has been suggested to directly interact with PKR to inhibit its activation, however, leaving the contribution of dsRNA-binding of A/NS1 on PKR inhibition unclear. Therefore, we performed a systematic analysis of basic amino acid residues in the RNA-binding domain of A/NS1 using reverse genetics. Mutating basic amino acid residues in A/NS1 without affecting the nuclear localisation signal, we generated 8 mutant viruses having 1 or 2 amino acid exchanges. Three mutant A/NS1 proteins demonstrated a defect in dsRNA-binding. While 2 of the corresponding mutant viruses showed a 10-fold reduction in viral replication on alveolar epithelial cells, the third mutant virus showed 10000-fold reduction and a diminished inhibition of PKR activation.
Thus, this study emphasizes the impact of dsRNA-binding of A/NS1 on viral replication. The effect of dsRNA-binding of the A/NS1 protein on PKR inhibition and other dsRNA-dependent cellular factors is currently under investigation.

The combined action of Influenza virus and *Staphylococcus aureus* Panton-Valentine Leukocidin provokes severe lung epithelium damage

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Keywords: S. aureus necrotizing pneumonia; pore-forming toxin PVL; superinfection with influenza virus

S. aureus necrotizing pneumonia is a life-threatening disease that is frequently preceded by an influenza infection. However the precise interplay between *S. aureus* and influenza virus remains to be elucidated.

In this study, we present a model that explains how influenza virus and the pore-forming *S. aureus* toxin Panton-Valentine-leukocidin (PVL) act together to cause necrotizing pneumonia. We found that PVL induced cell death in neutrophils, which was augmented by coinfection with influenza virus. Epithelial cells strongly up-regulated chemokine expression after influenza infection. Incubation of epithelial cells with supernatants from PVL-treated neutrophils resulted in cell detachment and disruption of the epithelial monolayer. Furthermore, intranasal instillation of mice with supernatants from PVL-damaged human neutrophils caused extensive airway epithelial exfoliation and tissue damage with signs of necrotizing pneumonia. The devastating effect on lung epithelium was completely prevented by adding protease inhibitors or human serum, indicating that destruction is caused by uncontrolled release of neutrophil proteases in the respiratory space. These findings can explain that necrotizing infections mainly develop in serum-free spaces such as pulmonary alveoli.

According to this model, modulation of influenza induced chemotaxis, PVL-induced neutrophil cytotoxicity as well as the uncontrolled release of proteases may represent possible targets for therapeutic interventions.

Comparison of glycoprotein-related pathogenicity functions of Lyssaviruses

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Keywords: lyssavirus, glycoprotein, pathogenicity

The Glycoprotein G of Rabies Virus (RABV) and other members of the genus Lyssavirus is decisive for receptor binding as well as virus entry by endocytosis and pH-dependent membrane fusion. Moreover, G is the major target for adaptive immune responses in all reservoir hosts. Accordingly, G is considered to play key role in host tropism and virus maintenance. For most Lyssaviruses tropism and maintenance are characterized by a strong bat-host restriction and only occasional spill-overs in non-bat hosts with onset of lethal rabies disease occur. To date, the mechanisms of host adaptation and virus-specific pathogenicity are not well understood.

To investigate the role of Lyssavirus G in tropism, adaptation and virus-specific pathogenicity, we compare G dependent effects on virus replication and pathogenesis in a genus-wide approach.

In-trans complementation of G deleted RABV revealed that even G proteins of phylogenetic distant Lyssaviruses were able to support RABV replication. Accordingly, chimeric RABV expressing G proteins from a broad panel of bat- and non-bat-borne Lyssaviruses were successfully rescued from recombinant cDNAs. Most of the envelope-switched viruses replicated to comparable titers in NA and BSR cells, confirming the compatibility of the heterologous G proteins with the RABV backbone. For certain G proteins, differences in cell tropism and cell-to-cell spread were observed in bat- and non-bat derived cells. Consistent with a lower virulence of European Bat Lyssaviruses Type 1 and 2 in mice, pathogenicity studies with the chimeric viruses in Balb/c mice revealed that G proteins from European bat Lyssaviruses were not able to confer pathogenicity factors to the RABV backbone. These data indicate that Lyssavirus G proteins are indeed a major factor in determining virus-specific pathogenicity.

Session Epidemiology, modelling and risk assessment II

12. October 2012
11:30 – 13:00

Room Ballsaal
Chair: Sandra Eßbauer

Multiple pathogen infections in rodents during a monitoring study in Germany

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Keywords: rodents, monitoring, zoonotic pathogens

Amongst wildlife species, wild rodents are considered the most important reservoirs for various zoonotic viruses, bacteria and endoparasites. The network "Rodent-borne pathogens" was established in Germany to study potential associations between rodent population dynamics and the prevalence of rodent-associated pathogens and their impact on the frequency of human infections.
During rodent monitoring in 2010-2011 at four selected regions in Mecklenburg-Western Pomerania (MWP), Thuringia (TH), Baden-Wuerttemberg (BW) and North Rhine-Westphalia (NRW) in spring, summer and autumn a total of 1826 small mammals were trapped. The majority of animals were represented by bank voles (n=1077), *Apodemus* mice (n=493) and *Microtus* voles (n=256).
Hantavirus investigations revealed the presence of bank vole-borne, humanpathogenic Puumala virus at the sites in BW and NRW. The humanpathogenic Dobrava-Belgrade virus was exclusively detected in MWP and TH, where striped field mice, the natural host of this hantavirus, were trapped. Interestingly, Tula virus was detected in *Microtus* voles from all four investigated regions. Initial molecular investigations demonstrated also the presence of *Leptospira* and *Rickettsia* spp., extended-spectrum beta-lactamase producing *Escherichia coli* and zoonotic *Giardia* assemblages.
Future investigations will have to prove the host association of the various pathogens, the frequency of spillover and simultaneous infections and the association of these findings with the rodent host population dynamics.

Antibody prevalence study for human pathogenic viruses in bats and rodents

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Keywords: Serology, small mammals, zoonosis

Bats and rodents are the most speciose and globally wide-spread mammalian orders. Both are reservoir hosts for a multitude of human pathogenic zoonotic agents. Virus isolation and nucleic acid detection both rely on timely sampling of viraemic animals shedding detectable amounts of virus. Serologic assays serve to identify long-term detectable antibodies and allow observing cross-reactivity with closely related viruses giving a far broader image on virus abundance in host species. In this study we screened 1136 bat (16 species) and 475 rodent (13 species) serum, blood or transudate samples by a mosaic chip-based immunofluorescence assay. We assessed antibody reactivity with 14 different viruses covering five virus families (Coronaviridae, Flaviviridae, Bunyaviridae, Paramyxoviridae, Togaviridae). The average bat sera reactivity ranged from 2% for Bunyaviruses to 44% for paramyxoviruses. In particular the African flying foxes *Rousettus aegyptiacus* and *Eidolon helvum* had cross-reactive antibodies against Yellow Fever virus (YFV) and Dengue 2 (up to 80%) and the paramyxoviruses Mumps and parainfluenza virus 2 (up to 89%). The overall rodent sera/transudate reactivity ranged from 1% for Alphaviruses and peaked for paramyxoviruses reaching 11%. This is the first global broad-range serologic survey indicating that bats and rodents have cross-reactive antibodies

against a multitude of human pathogenic viruses. The data can be used for targeted sampling of seropositive species.

Prevalence of antibodies against Tick-Borne Encephalitis Virus in wildlife in Saxony

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Keywords: TBE virus, wild boar, rodents

Currently Tick-Borne Encephalitis (TBE)-risk areas are defined by the Robert Koch Institute as those regions for which the average number of human TBE-cases is >1/100.000 per district in a five year period. Saxony is a state with a total of only 41 reported cases since 2001.
The aim of this study was to determine the true endemic areas in Saxony in order identify the areas of risk to acquire an autochthonous infection. This is usually done by flagging ticks and searching for TBE virus (TBEV) in them. Here we used an alternative approach by serological testing of wildlife. The rationale here was that roe deer and wild boars do seroconvert upon infection, they are heavily infested by ticks and usually have a home range which allows an allocation of a small geographic area.
913 sera of cloven-hoofed game were collected since April 2011 in several districts of Saxony. All sera were tested using Immunozym FSME IgG All Spezies ELISA and/or Anti-TBEV-IIFT IgG (IFA). A serum neutralisation test was conducted to confirm positive and questionable ELISA results.
Overall, 145 out of 913 (15.9%) of the sera had antibodies against TBEV. All samples that tested positive in the ELISA were confirmed positive by using the serum neutralisation test. This confirmatory testing is still pending for the sera that tested positive or questionable in the IFA.
Serosurveys in wildlife are useful in order to determine areas where TBEV is circulating in nature. However, the different levels of seroprevalence found did not correlate with number of human cases and thus cannot explain their occurrence. Hence, 85 rodents (usually having a smaller host rage) were trapped to identify supposed TBE endemic areas more precisely. Results of these investigations will be presented and discussed.

Diversity of *Bartonella* species in small mammals and their fleas in a recreational area in Leipzig, Germany

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Keywords: Bartonella, rodents, fleas

Bartonella spp. are facultative intracellular bacteria infecting erythrocytes and endothelial cells of its hosts. The cycle of these zoonotic pathogens includes different reservoir hosts such as small mammals and hematophagous arthropods such as fleas acting as vectors. Several rodent-associated *Bartonella* species have been linked to human disease, for example, *B. grahamii* and *B. elizabethae*. As data on the occurrence of *Bartonella* species in small mammals is scarce in Germany, DNA-extracts of spleen samples of small mammals (n=79, the majority bank voles and yellow-necked mice) and their fleas (n=135; from 43 individuals) from a recreational area in the city of Leipzig, Germany, were screened with a conventional PCR for *Bartonella* spp. followed by sequencing. Overall, 68.4% of the small mammals contained DNA of *Bartonella*: *B. grahamii*, *B. taylorii*, *B. doshiae* and DNA similar to *B. schoenbuchensis* and *B. birtlesii*. Double infections in individual animals were also detected. A total of five flea species was found on the small mammals: *Megabothris turbidus*, *M. walkerii*, *Ctenocephalus agyrtes*, *Doratopsylla dasycnema*, and *Nosopsylla fasciatus*. Altogether 59.3% of the fleas were positive for *Bartonella* spp. Sequencing of 44 revealed the *Bartonella* species *B. taylorii*, *B. grahamii*, *B. doshiae*, *B. elizabethae* and *Bartonella* spp. The public health impact of this finding of zoonotic *Bartonella* species in a recreational area requires further investigation.

Molecular typing of *Toxoplasma gondii* DNA detected in human samples from Germany

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Keywords: Toxoplasma, PCR-RFLP, genotype

Toxoplasma gondii is a protozoan parasite that infects several warm-blooded vertebrates, including humans. Wild and domestic felids shed the oocyst stage. Intermediate hosts such as humans, wild and livestock animals get infected by oral uptake of oocysts or infected intermediate hosts. In Europe, three clonal types of *T. gondii* (types I, II and III) are observed. While type I parasites are highly virulent, types II and III are less virulent in mice. Information on *T. gondii* types infecting humans in Germany is rare. Nine samples from patients (9/30; 30%) with ocular toxoplasmosis, 37/53 (70%) patients with congenital toxoplasmosis tested PCR-positive for *T. gondii*. Typing of *T. gondii* from three patients with ocular toxoplasmosis revealed type II alleles. The majority of congenitally infected patients were shown to contain *T. gondii* of type II (14/37, 41%), while two samples contained *T. gondii* of type III (1/37, 3%) and of a non-canonical type (1/37, 3%), respectively. This study shows that the majority of patients with toxoplasmosis in Germany is infected with type II *T. gondii.* In contrast, infection with *T. gondii* type III and non-canonical types is rare. The *T. gondii* types found in patients from Germany confirm previous data of genotypes found in cats and foxes in Germany.

This project is funded by the German Bundesministerium für Bildung und Forschung (01KI1002F) as part of the Toxonet02 Network (National Research Platform for Zoonoses).

Frequency and characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* from companion animals and horses

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Keywords: ESBL-E.coli, companion animals, fluoroquinolone resistance

The steady increase in the rates of invasive *E. coli* isolates resistant to 3rd gen. cephalosporins in humans has encouraged a discussion about the possible zoonotic transmission of antimicrobial-resistant (AR) bacteria. To determine how frequent ESBL-producing *E. coli* are present in samples from companion animals and horses and how similar they are to isolates from human patients, ESBL-*E. coli* were collected in a veterinary diagnostic laboratory from 2008 to 2010 and screened for resistance, virulence-associated and phylogenetic determinants.

ESBL-*E.coli* accounted for 3.2% of all (n=3858) *E. coli* isolates from cats, 4.0% from dogs (total *E. coli* = 7244), and 5.1% from horses (n=1344), while an increasing tendency could be observed over the time. CTX-M was the predominant ESBL-enzyme and those types identified (CTX-M-1, CTX-M-15, CTX-M-14) are frequently distributed among human isolates as well. Also the finding of (i) multilocus sequence types (ST), such as ST131, ST167, ST224, ST405, ST410, and ST648, which are likewise prevalent in human ESBL infections, (ii) comparable intra-ST macrorestriction patterns of human and animal strains, and (iii) identical fluoroquinolone resistance mechanisms supports the idea of a transmission of these bacteria between companions and humans.

As we have currently no clue for a host restriction of ESBL-*E. coli* in general or of certain genotypes, interdisciplinary and novel approaches in the spirit of “One Health”are even more important.

Session New and re-emerging zoonoses

12. October 2012
11:30 – 13:00

Room Steglitz
Chair: Thomas C. Mettenleiter

Chlamydia psittaci inclusion membrane protein IncB associates with host protein Snapin to interact with the host microtubule network

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Keywords: chlamydial inclusion, dynein, Inc proteins

Chlamydia (C.) psittaci comprise a group of obligate intracellular prokaryotes that cause a variety of diseases in birds, being also pathogenic to man. During the unique developmental cycle, the elementary body, which is the infectious form, gains access to the susceptible host cell where it transforms itself into the reticulate body, the multiplicative form. All this processes take place within a parasitophorous vacuole called inclusion, which is modified by bacterial type III secreted effector proteins, termed Incs.
Here we describe the interaction between the inclusion membrane protein IncB of *C. psittaci* and the host protein Snapin in a yeast two-hybrid system. Snapin is a cytoplasmic protein with a multivalent role in intracellular traffic events. In GST-pull down experiments with 35S-labelled proteins and cell lysates both *in vitro* and *in vivo* interaction between full-length IncB and Snapin were shown. Using confocal fluorescence microscopy and an IncB-specific antibody, the co-localization of IncB, Snapin and the microtubule (MT) motor protein dynein near the inclusion membrane of *C. psittaci*-infected Hep-2 cells was demonstrated. MTs have been proposed to be involved in the chlamydial redistribution from the host cell surface to the perinuclear region, but little is known, how the different components of this eukaryotic structure interact with Chlamydia.
We hypothesize that Snapin connects chlamydial inclusion with MT network by interacting with both IncB and dynein.

The novel family *Mesoniviridae* as a potential model of virus emergence

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Keywords: insect nidovirus, mesonivirus, dilution effect

Recently we discovered a novel RNA virus family in mosquitoes (Zirkel et al, mBIO 2011). The prototype Cavally virus (CAVV) now defines the family *Mesoniviridae* within the order *Nidovirales* (including, e.g., the coronaviruses). CAVV was isolated in the area of the Taï National Park, Côte d'Ivoire, and was shown to emerge from a pristine rainforest to human settlements. Critically, virus diversity decreased and prevalence increased along a habitat modification gradient out of the forest. This overall observation reflected a dilution effect as known in organismic ecology. The ecology of *Mesoniviridae* may be paradigmatic for the emergence of novel arboviruses through tropical ecosystems destruction.

This presentation summarizes ecological results along with a significantly intensified investigation of the novel family. Three further species were detected at overall prevalences of 0.23%, 0.69%, and 7.63% in mosquitoes. Genomes were 59-89 % identical on nucleotide level and 48-85 % on amino acid level. Six open reading frames were conserved in all viruses. Several smaller and less conserved ORFs were detected at the 3' end of the genomes, putatively coding for accessory proteins. Unique among nidoviruses, mesoniviruses use two distinct leader body fusion sites for their sgRNA synthesis. Seven proteins were identified by western blotting and Edman sequencing, mapping to ORF2a, -2b, and 3a. These data will enable the study of mesonivirus replication, host interactions, and ecology.

Establishment of a new H1N2 lineage of swine influenza viruses in Germany

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Keywords: swine influenza A virus, pdmH1N2 reassortant, H3pdmH1

In March 2009, influenza A (H1N1) pdm09 virus (pdmH1N1) emerged after reassortment of a North American triple reassortant and the European avian-like swine H1N1 virus. This pdmH1N1 is well-adapted to humans and pigs and readily replicates in both hosts. In order to investigate the incursion of this virus in German pigs, some 1800 swabs of diseased pigs were tested by PCR for pdmH1N1 in 2009-2011. 423 samples were influenza A virus positive, 14 of which were positive for pdmH1 and/or pdmN1 (3.3%). So far, virus isolation was successful for 9 samples of 8 different herds. Sequencing of their genomes revealed two novel reassortants. The pdmH1N2 reassortant was characterized by a hemagglutinin gene of pdmH1N1 virus whereas the neuraminidase gene was derived from the human-like H3N2 lineage prevalent in European pigs. This reassortant emerged in 2010 in Germany and still persists indicating a stable infection chain within the pig population. Phylogenetic trees comprising available sequences of pdmH1N1 and pdmH1N2 strains demonstrate at least eight other distinct genotypes of H1N2 reassortants with genes of the pdmH1N1 that have been isolated in Korea, USA, England, Italy and Germany. These reassortant strains comprise genes of the pandemic, seasonal human and porcine lineages. The other reassortant, H3pdmN1, has not been described so far. The pdmH1N2 and H3pdmN1 reassortants pose a significant zoonotic risk for humans with occupational exposure to pigs and their family members.

Vector competence of German *Culex* species for West Nile virus

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Keywords: Culex spp., West Nile virus, Vector competence

West Nile virus (WNV) has caused repeated large-scale human epidemics in North America since it was first detected in 1999. In North America, vectors for WNV are mosquitoes of the *Culex* genus such as *C. pipiens* sensu stricto and *C. p. pipiens* biotype molestus. These species are highly abundant in Germany and raise the question, if German mosquitoes could be vectors for WNV. In addition, human WNV infections were already reported from Italy and Hungry. Thus, making a carry-over of the infection to Germany likely. In our vector competence study, we address I) the vector competence of German *Culex* spp. mosquitoes for WNV and II) the distribution of competent mosquito species in Germany. As a reference system we chose *C. pipiens quinquefasciatus*, which has been associated with WNV transmission in the United States . Using the reference strain, a German *C. pipiens pipiens* biotype molests culture strain and *Culex* spp. mosquitoes collected in the wild from different regions in Germany were analyzed for the infection rates with WNV. We will further analyze if the geographic origin of the virus isolate and the number of passages has an influence on infection rates, by using WNV Serotype 1 isolates NY99, Egypt 101 and Italy.

Medical importance of Usutu virus in south-west Germany

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Keywords: Usutu virus, Germany, blood donor

Usutu virus (USUV) is a mosquito-borne, single-stranded RNA virus and belongs to the *Japanese encephalitis* virus group within the family *Flaviviridae*. After the initial detection of USUV in German mosquitoes in August 2010, the virus spread in 2011 and caused epizootics among wild and captive birds in south west Germany. Therefore, we initiated this study to investigate the medical importance of USUV in an area with high USUV infection rates in mosquitoes and birds. 4200 serum samples from healthy blood donors from south-west Germany were collected in January 2012 and analyzed for the presence of USUV-specific-IgG antibodies with an in-house indirect IFA and a commercial ELISA. Positive tested serum samples were further investigated for serological cross-reactivity to other flaviviruses (West Nile virus and Tick-borne encephalitis virus) by endpoint titration in IFA. In addition, samples were further investigated with virus neutralization assays (VNTs). 84 serum samples originating from different healthy blood donors were tested positive in IFA and ELISA. Serological cross-reactivity with other flaviviruses was observed and highest endpoint titers against USUV were demonstrated for 8 samples. However, all 84 samples will be analyzed in the VNTs in order to demonstrate the presence of USUV-specific neutralizing antibodies. In conclusion, Public health authorities, blood transfusion service and clinicians in Germany should be aware of the risk of USUV infection in humans.

Susceptibility studies with rabies virus strain isolates in German raccoons (*Procyon lotor*)

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Keywords: raccoon, rabies, susceptibility

Raccoons, the most frequently reported rabid animal species in the USA, can be found almost everywhere in Central Europe since their first successful introduction in 1934. However, Germany is the only European country with well established populations, with especially in (semi-) urban areas extreme high population densities (approx. 100 animals/km²). Interestingly, during the last European fox rabies epizootic only few rabid raccoons were reported from Germany. Most likely the population density of raccoons was in these years still relatively low. However, other factors like low susceptibility to the fox rabies (RABV) virus variant and low genetic diversity among German raccoons could also have played a role. To investigate the susceptibility of the German raccoon population, wild caught animals were inoculated with the most likely lyssavirus variants to infect the local population. It was shown that the German raccoons were fully susceptible for a dog and raccoon RABV variant. Five of 6 raccoons inoculated with a fox RABV isolate showed subsequently clinical signs. However, none of the infected raccoons succumbed to rabies after infection with European Bat Lyssavirus Type 1 (EBLV-1); although all these raccoons seroconverted. Thus raccoons could become a new reservoir species for rabies upon re-emergence of terrestrial rabies in Germany.

Poster presentation

Poster Session Epidemiology, modelling and risk assessment

E 01

Overview on the existence of integrative information systems for zoonotic disease surveillance

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Keywords: zoonotic disease surveillance, one health, integration of different data sources

Even though 65% of recent major disease outbreaks have zoonotic origins, there is still a sharp division of the disciplines in human and veterinary health. In the last decade, a global integrative concept, often referred to as “One Health”, has been strongly endorsed. In the course of its adoption in Germany, claims were raised to enable a cross-sectoral data interpretation of zoonotic disease information in different epidemiological databases, applying the requirements of secondary data analysis. It is assumed that this will improve the prevention, prediction and control of zoonoses.

To provide an overview of existing projects throughout the world that integrate information from humans and animals on zoonotic diseases, a literature search was conducted. Projects found were categorized and described regarding their concepts and realization.

108 projects were identified at all. 18 systems integrate MOSS data from humans and animals on zoonotic diseases from different sources. These projects either analyse health related, often unstructured data from non-traditional sources (n=5) or high aggregated data and condensed information from experts (n=5) or raw or less aggregated data from official sources to describe level and distribution or changes in disease occurrence (n=8).

Their approaches are diverse, so the feasibility of the stated claims in Germany has to be conducted on an individual level and closely fitted to the demands of the different stakeholders.

E 02

Expert judgement elicitation procedure to gain knowledge about the risk pets pose to their owners' health

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Keywords: expert elicitation, pets, zoonotic risks

More than half of all human notifiable infectious diseases are zoonoses. Since more than 25% of German households own pets, among these about 8.2 million cats and 5.4 million dogs, infectious diseases of animals may pose a risk to human health.
Until now, the extent of this risk has not been exactly examined or quantified in Germany. Therefore it is the aim of this project to calculate the risk for dog and cat owners to be infected and sickened.
Carrying out an intensive literature research, pathogens were identified (bacteria, virus, parasites) that are transmittable from cats and dogs to humans.
Prevalences of these pathogens in the pet population are estimated on the base of studies from Germany and neighboured countries with comparable living and housing conditions.
Experts from different fields (human medicine, veterinary medicine, research) are asked to rate the probability of an infection and sickening of the owner if his cat or dog is carrier of the pathogen. In addition, veterinary and health authorities will be consulted to validate the background of veterinary public health.
The risk of disease can be ranked as high, rather high, medium, rather low or negligible and is assessed for dog and cat owners as well as for children (up to 12 years) and adults (12-60 years) separately.
Transmission ways of pathogens and the access of the animals to the living space of the owner are defined in advance (out-door cats and dogs with access to the bedroom).
On the base of the risk of disease stated by the experts and the prevalences, the infectious risk for dog and cat owners for each pathogen is assessed and the overall infection risk over all pathogens is investigated.

First results will be presented to contribute to the prioritisation of the infectious risk posed by pets.

E 03

Bat rabies surveillance in Europe

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Keywords: European bat lyssaviruses, bat lyssavirus surveillance,

To date, four lyssavirus species have been isolated from European bats: EBLV-1, -2, BBLV and WCBV. Unlike in the New World, bat rabies cases in Europe are comparatively less frequent. Thus the objective was to assess bat rabies surveillance in Europe, taking into account official data of the WHO Rabies Bulletin Europe (RBE) and published studies. In Europe, 959 bat rabies cases were officially reported (1977-2010). The vast majority was characterized as EBLV-1 (from *E. serotinus, E. isabellinus*) followed by a few occasional isolations of EBLV-2 (*M. daubentonii, M. dasycneme*), and a single BBLV isolation from *M. nattereri*. A total of 3880 oral swabs from 23 different European bat species have been screened during active surveillance, and only few samples from Eptesicus bats (EBLV-1, N=49) and Daubenton´s bats (EBLV-2, N=2) yielded viral RNA. This study shows that the level of bat rabies surveillance in Europe is still very heterogeneous, despite international recommendations. Raising awareness, establishment of efficient networks of bat biologists, additional testing of moribund bats, identification of lyssaviruses and bats to species level, and reporting to the RBE are key elements to enhance bat rabies surveillance in Europe. Active surveillance adds useful information regarding the dynamics of infection in natural populations. However, bat serology needs standardisation to better understand the meaning of lyssavirus specific antibodies as detected in various European bat species.

E 04

Serological surveillance of morbilliviruses, influenza viruses, toxoplasma and brucella in dolphins in Russia.

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Keywords: Marine mammals, antibodies. surveillance

Marine mammals can be a reservoir for phylogenetically diverse pathogens belonging to different biotypes that can evolve for a long time to accumulate and persist in nature. Holden (1972) was probably the first who suggested to use marine mammals for monitoring of the ecology and environment. Marine mammals are a good indicator for medium-and long-term observations of changes occurring in the environment, since many of them live long enough to occupy the top of the food pyramid [Aguilar, Borrell, 1994]. In this work the presence of antibodies to morbilliviruses (CDV), brucella, influenza virus (H1, H3, H4, H13 subtypes of influenza viruses isolated from birds, H1, H3, H7 subtypes of influenza virus isolated from mammals) and Toxoplasma was Studied in the populations of Black Sea bottlenose dolphins and beluga whales, living in the Sea of Okhotsk. Samples from 74 of Black Sea bottlenose dolphins and 248 beluga whales of the Okhotsk Sea were collected in 2002-2010 immediately after capture. The level of antibodies to morbilliviruses, toxoplasmosis, and brucellosis was determined with a commercial enzyme immunoassay antigen. Antibodies to influenza A viruses were determined using the hemagglutination - hemagglutination inhibition reactions.

Morbillivirus-specific antibodies were detected in 15 (20.3%) bottlenose dolphins and 32 (12.9%) belugas. The number of Brucella positive samples was 17 (23.0%) in bottlenose dolphins and 17 (6.8%) in beluga whales. Antibodies to Toxoplasma were detected in the serum of 39 (52.7%) bottlenose dolphins and 14 (5.6%) belugas. No antibodies to influenza viruses were detected.

The high presence of infection in marine mammals was observed near highly populated and economically developed coastal area.

E 05

Evidence of polyomavirus transmission between wild living chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in Loango National Park, Gabon

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Keywords: wild apes, microorganism transmission, polyomaviruses

Wild-living primates may carry microorganisms which have zoonotic potential. Though surveillances have been focused on areas where "human-primate contact" is frequent, it is not clear to what extent microorganisms readily cross "species barriers" in primates inhabiting pristine areas.

In Loango National Park, Gabon, gorillas and chimpanzees, both genetically closely related to humans and to each other, live in sympatry. We used Loango apes as a model for microorganism transmission between wild hominines by performing a broad microorganism screening on fecal samples collected from these primates (ca. 60 each). Targeted microorganisms (simian foamy virus, enterovirus, plasmodidae, bocavirus, polyomavirus (PyV)) were chosen to represent many routes of transmission.

Most exhibited no or very low prevalence in both species, with PyVs standing as the exception. Three distantly related PyVs were identified in chimpanzees and one in gorillas using a generic PCR approach. Consecutively, PCRs targeting specific PyVs detected in each of the two species were designed and samples re-screened. The latter revealed likely transmission of at least two groups of PyVs between wild chimpanzees and gorillas. This highlights the possibility of PyVs interspecies transmission and points at the zoonotic potential of these viruses.

E 06

New aspects of epidemiology and molecular characterization of Tick-Borne Encephalitis Viruses in Bavaria

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Keywords: Flaviviridae, TBEV, Ixodes ricinus

Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family Flaviviridae and is transmitted by ticks. Three subtypes in Europe and Asia have been distinguished. In Central Europe, so far only TBEV of the Western subtype has been detected. The dynamics of the transmission of TBEV in natural foci is poorly understood. The aim of our study is to better understand the transmission dynamics in natural foci and of the spread of TBEV in Bavaria, Germany.

The abundance of ticks and of TBEV in different TBE foci is tested on the basis of the appearance of human cases. Since 2009, *Ixodes ricinus* ticks have been sampled in monthly intervals in a standardized way. Ticks were tested by RTQ-RT-PCR in pools. The E-genes from positive TBEV ticks are sequenced and compared to other available sequences.

The highest numbers of ticks has been detected in the months May and June of the respective years. TBEV infection rates in the different tick stages differed during the years. 39 of 14903 ticks were tested TBEV positive in RTQ-RT-PCR. The comparison of the E-Genes shows some genetic heterogeneity and several genetic clusters of TBEV could be distinguished within one single natural focus. Our data also imply multiple and continuous introductions of TBEV strains from Eastern Europe into Germany.

Our data show the TBEV spread in small foci in Bavaria. Further studies will be necessary to help for a better understanding in the dynamics of TBEV spread in Germany.

E 07

Influenza A virus as a zoonotic infection

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Keywords: Influenza, zoonotic infections

Avian influenza is a highly contagious disease caused by Influenza viruses (Orthomyxoviridae family). Main natural viral hosts are wild waterfowls, especially birds of the orders Anseriformes and Charadriiformes. Seasonal wild bird migration flights promote virus distribution over long distances. The viruses were also isolated from other bird species, mammals (swine, horse, dog, cat, whales, etc.) and even human. All viral subtypes (16 subtypes of hemagglutinin (HA) and 9 subtypes of neuraminidase (NA)) were isolated from waterfowls and all these subtypes are low pathogenic (LP). But some viruses of H5 and H7 subtypes can cause highly pathogenic avian influenza (HPAI).

During surveillance program for avian influenza viruses in Russia our team isolated more than 250 viruses since 2002. The pathogen was detected in wild bird species, poultries, swine, seal, and muskrat. Most of the viruses are LP. But during the outbreak of HPAI H5N1 viruses in Russia in 2005, we also isolated some HPAI strains from wild waterfowls and poultries. Since that time, we continually detected HPAI H5N1 viruses in wild birds in Russia.

Influenza viruses can easily transmitted from one species to another causing risk to format a pandemic or epizootic viral variant. This fact was confirmed by two last outbreaks – "bird" flu (2005) and "swine" flu (2009). Thus, influenza viruses can cause not only economic loss, but also be a serious threat for human health.

E 08

Q fever outbreak in humans after a caesarean of a goat

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Keywords: Coxiella burnetii, goats, zoonosis

At 4th Sept. 2009 a cashmere goat was admitted to the clinic due to dystocia. A caesarean was performed and 2 dead foetuses were delivered. The placenta was tested positive for *Coxiella burnetii* by PCR. All members of the staff and students which had participated at the caesarean were informed immediately about the circumstances and pointed towards a possible human Q fever infection. Six of the 8 directly involved persons showed an antibody titre after 2 to 8 weeks. Only 4 of these were indicative for a recent Q fever infection. These 4 individuals showed mild to severe clinical signs as flue-like symptoms, 2 were hospitalized, between two and five weeks after the exposure to the infected goat.

Moreover, the whole goat flock (18 animals) was sampled on 9th Sept. 2009. All of the vaginal and preputial swabs taken were tested positive by PCR. Seven of 8 female goats, 4 of 5 bucks as well as 2 of 3 lambs were serologically positive. Two additionally purchased animals were serologically negative. Control measures as well as Coxevac® vaccination and oxytetracycline treatment of the goats were ordered based on the "Infektionsschutzgesetz" for human safety.

In this case, the early identification of the *Coxiella burnetii* infection of the goat led to a targeted and early treatment of Q fever of the diseased patients. This emphasises the importance of a rapid communication and collaboration between veterinarians and physicians.

E 09

Monitoring of an urban pigeon population reveals a high prevalence of *Chlamydia psittaci* and also highlights non-classified chlamydiae

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Keywords: Chlamydia psittaci, urban pigeons, DNA microarray testing

Urban pigeons are known carriers of chlamydiae and represent a possible reservoir for zoonotic transmission to humans. A number of recent epidemiological studies has provided sporadic evidence on the epidemiological situation. However, studies of *Chlamydia (C.)* infection in birds have usually been confined to the search for *C. psittaci*, so that little is known about the occurrence and identity of other chlamydial agents. In the present study, cloacal swabs and faecal samples of urban pigeons have been examined by real-time PCR, DNA microarray assays for species identification and ompA genotyping, partial 16S rDNA and ompA sequencing, as well as cell culture.

While *C. psittaci* proved to be the predominant chlamydial agent (75.8 % of all *Chlamydiaceae* positives), atypical serovars and other species, such as *C. abortus*, *C. pecorum** and *C. trachomatis*,* were also present (*first description in avian host). Rather unexpectedly, 19.5 % of all *Chlamydiaceae*-positive cases turned out to be due to non-classified organisms of the same family. The identification of this diverse spectrum of chlamydial strains and species became possible because of the combined use of highly specific and sensitive molecular assays. The considerable prevalence rate of novel agents raises the question of their epidemiological importance and possible role as pathogens. Future surveys in domestic and wild birds will have to take the extended variety of chlamydial organisms into account.

E 10

Recording of epidemiological study data on prevalence of MRSA using a systematic database

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Keywords: MRSA, meta-analyis, data base

Prevalence is one fundamental parameter in population epidemiology. Data on prevalence are necessary to judge the hazard of an infectious disease, to plan surveillance and control measures and these data are the basis of mathematic models concerning the spread of a disease.

For many infectious diseases data on prevalence are not available from national or international surveillance systems and the situation becomes even worse if both, animal as well as human populations are of interest. Published data from scientific studies have to be used in this situation, so to obtain a comprehensive overview of the existing data, a systematic literature review is necessary.

In a current investigation a systematic review on studies concerning the prevalence of MRSA in pigs and in humans with pig contact is conducted. The data which are collected in these predominantly observational studies show a great variety which is a challenge for data management and analysis.

To overcome this problem a data base was designed and evaluated. This data base covers the following fields:

- The study itself (Who? Where? When?)
- The population under study
- The sampling method
- The laboratory method
- The isolate characteristics
- Resistance of isolates to antimicrobial substances
- Bias and limitations

Although this approach was developed for the outcome MRSA it could also be applied for prevalence studies of other pathogens in zoonoses research.

E 11

Prevalence of MRSA nasal colonization over time in veterinarians and their household contacts in Germany

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Keywords: LA-MRSA, Zoonosis

Carriage of livestock-associated (LA-)MRSA is common in exposed humans. We report on nasal colonization with LA-MRSA in veterinarians, dynamics over time and spread to their contacts.
We used a cohort of veterinarians (7.7% MRSA pos.) from studies conducted in 2008-09. A case was defined as a former study participant with MRSA-positive nasal swab; eligible controls were MRSA-negative. All cases were contacted and compared to 2 controls per case. Respondents and household contacts (HC) received questionnaires and material for self-swabbing. Retesting was offered to cases that tested MRSA-negative. We described background data and conducted comparative molecular typing of isolates.
We included 74 cases and 151 controls. MRSA was detected in 42 cases (56.8%), 38 carried spa types identical to 2008-09, 69.0% carried ST398. Retesting of MRSA-negative cases detected 4 additional ST398. In 9/151 (6.0%) controls MRSA was detected: 7 ST398, 1 ST254 and 1 ST97.
Among 141 HC of cases, 18 (12.8%) were MRSA-positive; all carried the same clonal complex as the associated case (17 ST398, 1 ST225), 77.8% the same spa type. One/251 (0.4%) HC of controls was LA-MRSA positive.
Identical spa types in one household indicate intrafamilial spread, although risk exposures await evaluation. Our study suggests prolonged exposure time to LA-MRSA at home is a risk factor. HC of known LA-MRSA carriers might thus be considered when defining risk groups for preventative measures.

E 12

Proportion and genetic background among MRSA from wound swabs of companion animal origin in Germany

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Keywords: MRSA, companion animals

Staphylococcus aureus is an important opportunistic pathogen in human medicine and in particular infections with methicillin resistant *S. aureus* (MRSA) lead to high costs in the human health care system. MRSA transmission is well described between different hosts and several reports revealed that above all MRSA infections in companion animals are frequently caused by "human-associated" lineages. However, information is scarce about the proportion and impact of these infections.

To gain information about the distribution of *S. aureus* in wounds of companion animals, 3,479 clinical swabs from dogs, 1,146 from cats and 604 from horses were screened for the presence of *S. aureus* respectively MRSA. The German wide study was carried out between November 2010 and March 2012 and *S. aureus* was identified in 5.8% (n=201), 12.2% (n=140) and 22.8% (n=138) of the canine, feline and equine wound swabs respectively. The MRSA proportions ranged from 3.6% (n=126) in wound samples from dogs, 5.7% (n=65) in those from cats, to 9.4% (n=57) in those from horses.

S*pa* typing and PFGE analysis was performed for all MRSA revealing a comparable distribution of clonal complexes (CC) among canine and feline strains with CC22 (c.: 51.2%, f.: 50.8%) and CC5 (c.: 30.6%, f.: 30.2%) as predominant lineages. These genotypes represent frequent lineages in human infections as well.

In contrast, the majority of wound infections from horses were caused by CC398-associated MRSA, belonging to the group of livestock-associated (LA-) MRSA.

E 13

Emergence of the novel *mec*A homologue among MRSA of companion animal origin in Germany

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Keywords: mecA homologue, animal, MRSA

Infections due to methicillin resistant *Staphylococcus aureus* (MRSA) are among the most difficult to treat in humans, as well as in the field of veterinary medicine. The general transferability of MRSA between different hosts, including humans, dogs, cats and horses as well as livestock animals is an increasing concern for epidemiologists worldwide.

Recently, a novel *mec*A homologue (*mec*A_{LGA251}; NCBI FR821779.1) was described for MRSA isolates from human and ruminant hosts.

Here we report about the occurrence of the novel MRSA variant in clinical specimens from dogs (n= 2), cats (n= 7) and a guinea pig in Germany. Genotypic characterization by use of microarray hybridization, multilocus sequence typing (MLST) and *spa* typing revealed that four strains belonged to ST130 and one to ST1945. The *spa* types associated with these STs of the clonal complex (CC)130 were t843, t1773, t10009 and t10033. The remaining five isolates were assigned to ST599 and the detected *spa* types were t1694, t278 and t10006.

As reported before, most of the MRSA isolates showed a low or moderately high oxacillin minimum inhibitory concentration (MIC) between 0.5 and ≥4 µg/ml.

The detection of the novel MRSA homologue among clinical isolates from companion animal origin underlines once more the importance to consider the ability of *S. aureus* to adapt to different host species in order to gain insights into the general epidemiology of *S. aureus*, including MRSA and their zoonotic impact.

E 14

Characterization and risk assessment of MRSA from food

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Keywords: MRSA, food, risk assessment

Livestock-associated Methicillin-resistant *Staphylococcus aureus* (la-MRSA) have been described with varying prevalences at primary production and along the different food chains in several countries, including Germany. Working or living on a farm was proven to be a risk factor for acquiring MRSA, whereas the exposure risk decreases from farm to fork and was considered to be low for food.
Since 2009, more than 1.500 MRSA isolates from various food samples (raw meat from pork, veal, beef, game, turkey and chicken) and taken in the course of the national monitoring of zoonotic agents were intensively characterized at the National Reference Laboratory for staphylococci including *Staphylococcus aureus* at the BfR. All isolates were multiresistant against at least 3 different classes of antimicrobials. The majority of MRSA (85.2 %) belonged to clonal complex CC398, with t011 and t034 being the predominant *spa* types. Among isolates from poultry, non-CC398 MRSA of *spa* types t1430 (chicken) resp. t002 (turkey) were more frequently detected.
Only few isolates carried genes encoding for one of the well described virulence determinants (e.g. enterotoxins, leukocidins etc.). Nevertheless, findings of Panton-Valentine leukocidin (PVL) positive MRSA in food warrant special attention and need to be considered in risk assessment.

E 15

Severe infections of skin and soft tissue (SSTI) in humans with LA-MRSA ST398

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Keywords: LA-MRSA, ST398, zoonotic infection

SSTI such as furunculosis, abscess, whitlow are most often associated with *S.aureus.* In relation to numerous clonal lineages of which the population structure of this species is composed, only a few of them are able to cause SSTI: MSSA of clonal complexes CC30 and CC121, CA-MRSA attributed to CC5, CC8 ("USA300"), CC22, ST59, ST80, and finally also LA-MRSA ST398 which is not only a frequent nasal colonizer of humans exposed to livestock. From 2008 tom 2010 it represented 12 - 15 % of MRSA isolates from SSTI (CA-MRSA, ST8: 21 %, ST30: 12 %, ST80 24 %, others 28%)
Different from CA-MRSA , LA-MRSA ST398 don`t produce PVL, the isolates investigated so far are also negative for the immune evasion gene cluster (*sak, chp, scn*) which is usually contained by *int*3-type prophages. With respect to demonstration of virulence associated genes by means of a DNA-microchip (Alere) LA-MRSA ST398,V, from SSTI are not different from isolates derived from nasal colonization of livestock and humans.
Conclusion: LA-MRSA ST398 obviously possesses and initial patho-potential for SSTI in humans.

E 16

Molecular and microbial tools for the risk assessment for the zoonotic potential of *Streptococcus gallolyticus* subsp. *gallolyticus*

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Keywords: Streptococcus gallolyticus subsp. gallolyticus, new zoonosis, endocarditis

Analysis of infected human heart valves revealed a significant relevance of *Streptococcus gallolyticus* subsp. *gallolyticus* (formerly *S. bovis* biotype I) as a causative agent of infectious endocarditis. This bacterium can be identified as a commensal and pathogen in humans and animals, including mastitis, sepsis and infections in various animals. Furthermore several studies showed an association between colorectal cancer and *S. gallolyticus* subsp. *gallolyticus* as causative organism.

The knowledge of the transmission of this pathogen is still limited. In order to analyze the prevalence of this pathogen in the human gastrointestinal tract and several animals, a real-time PCR for the distinct detection of *S. gallolyticus* subsp. *gallolyticus* was established. For screening and isolation of viable strains, selective culture conditions were established, allowing the isolation out of several materials (e.g. human and animal faeces). Furthermore an ERIC-PCR, a rep-PCR and for the first time a *S. gallolyticus* subsp. *gallolyticus* specific MLST (multi-locus-sequence-typing) scheme was established.

First results, considering strain collection isolates, showed no evidence for host or geographic related specific clustering. This indicates that a transmission between human and animal cannot be excluded. The development of detection, isolation and typing methods provides the basics for analyzing the epidemiological connections, infection chains and a systematically screening.

E 17

Prevalence and molecular characterisation of *Clostridium difficile* in companion animals and their owners

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Keywords: Clostridium difficile, enteritis, zoonosis

Clostridium (C.) difficile infection (CDI) symptoms range from mild diarrhoea to life threatening pseudomembranous colitis. As a cause of nosocomial enteritis *C. difficile* accounts for high costs due to prolonged hospitalisation and intensified medical care. The question whether companion animals may present a natural reservoir for virulent *C. difficile* strains affecting humans is addressed in this study. This study aims to collect first national data with regard to the prevalence and occurring ribotypes in dogs, cats and their owners. Risk factors which are associated to the acquisitions of CDI or colonisation will be investigated.

It is planned to collect and examine faecal samples from patients (dogs and cats) and their owners at the small animal clinic of the FU Berlin. The isolated *C. difficile* strains will be typed (ribotyping by capillary-gel-electrophoresis, PCR detection of toxin gene A and B as well as the binary toxin gene). Data concerning the animals and their owners will be collected using a questionnaire designed within this project, evaluated statistically and compared with data collected from human medicine.

This study should enable a first estimation of the possible zoonotic potential of *C. difficile* in companion animals.

E 18

Occurrence of Shigatoxin-producing *Escherichia coli* (STEC) in German Petting Zoos

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Keywords: E. coli, petting zoo, STEC

Cases of clinical disease caused by Shigatoxin-producing *Escherichia coli* (STEC) in humans are reported in increasing numbers. STEC can cause bloody and non-bloody diarrhea as well as hemolytic uremic syndrome (HUS), especially in children. STEC infections in humans are mostly associated with consumption of raw milk or raw meat. Close contact to ruminants was described as another source of infection. Therefore, in 2008 and 2009 we have performed a pilot study on the occurrence of STEC in German petting zoos. A total of 54 petting zoos had participated in the study, and in 27 of them (50%) STEC could be isolated. Overall we detected 33 different serotypes, and up to 13 different serotypes within one zoo. The isolated strains originated from goats, sheep, pigs and cattle.

In 2011 we started a follow up study to find out whether STEC strains in previously positive petting zoos might be endemic. Nine of the previously positive zoos agreed to participate again. These were sampled quarterly over a period of one year. Results of the (ongoing) study showed that all tested petting zoos remained STEC positive. Currently, the isolated strains are being typed by classical serotyping as well as MLST in order to determine their homology to previously isolated STEC strains. PFGE and sequencing is then planned to be done with possibly identical clones/strains.

Taken together, our results show that STEC occurs in German petting zoos and suggest that STEC might be endemic in such zoos.

E 19

High fecal carriage rates of ESBL-*E. coli* in urban Norway rats.A threat for public health?

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Keywords: ESBL, rats

Initial reports give evidence for the occurrence of ESBL-producing *Escherichia coli* in feces of urban rats. To get first insights into possible transmission pathways of multiresistant strains in urban environments we compared ESBL isolates from wild rats with human clinical isolates from the same area. A total of 56 wild Norway rats from Berlin (Germany) originating from the sewage system or trapped in buildings were therefore screened for ESBL-producing *E. coli.* Additionally fifty human clinical ESBL isolates from a university hospital nearby the trapping sites were compared to the rat strains.
From 80% of the rats (n=45) we were able to isolate *E. coli* and after exclusion of double clones via Pulsed-field gel electrophoresis 42 *E. coli* strains were screened for ESBL production. Multilocus sequence typing and structure analysis followed to determine the phylogenetic background of ESBL producing *E. coli.*
Sixteen percent of all rats (n=9) carried an ESBL-*E. coli* (mostly $bla_{CTX\text{-}M\text{-}1}$) with even higher rates for animals obtained from the sewage system (33%). Additionally, MLST of the ESBL-*E. coli* revealed sequence types like ST410 and ST90 that have also been found in clinical ESBL-*E. coli*. Nevertheless, whilst comparing the clonal relatedness of rat and human clinical samples we did not detect direct clonal relatedness. This might indicate a plasmid borne spread of ESBL-genes instead of direct clonal spread, or might be influenced by the small sample number. The plasmid characterization of the strains is still ongoing, but even unaware of this results the high carriage rate ESBL-*E. coli* in urban rats might result in additional negative implications for public health caused by these urban pests.

E 20

Eating ESBLs – modeling the exposure to ESBL-producing *E.coli* via the broiler meat chain

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Keywords: ESBL, broiler, exposure assessment

During the last years a growing rate of antibiotic resistant bacteria could be observed in livestock animals. Especially concerning is the rising rate of enterobacteria which produce extended spectrum beta-lactamases (ESBLs). ESBL-producing bacteria can not only destroy penicillins but also cephalosporins of the 3rd and 4th generation. In 2009, the BfR investigated *E. coli* and *Salmonella* isolates from broiler meat and found that approximately 5 to 6 percent were resistant to cephalosporins. The joint research project RESET aims at investigating the origin and the transmission pathways along the food chain of ESBL-producing *E. coli* and the assessment of potential risks for humans. As part of a risk assessment framework for ESBLs we developed a concept for modeling and quantifying the exposure of the consumer with ESBL-producing *E. coli* via the broiler meat chain. The exposure model incorporates concepts designed by Nauta et al. (2007) for modeling the transmission of *Campylobacter*. It models the changes in pathogen numbers along the steps of the broiler food chain, starting with entrance into the slaughterhouse and ending with the consumption of cross-contaminated salad by the consumer. The authors intend to present their modeling framework and give first insights into the parametrization of the exposure model and its first results.

References:

Nauta, M.; Jacobs-Reitsma, W. & Havelaar, A. A risk assessment model for Campylobacter in broiler meat *Risk Anal,* 2007*, 27*, 845-861

E 21

Source attribution of foodborne ESBL-*E. coli* in Germany

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Keywords: Source attribution, model, ESBL-producing E. coli

Extended-spectrum beta lactamases (ESBL) are enzymes able to inactivate a wide range of important beta-lactam antibiotics including penicillins, cephalosporins and monobactams. Therefore, ESBL-producing bacteria (e.g. *E. coli*) are multidrug resistant and the treatment of infections in animals and humans is more and more demanding.

The German joint research project RESET aims at closing knowledge gaps concerning the origin and the transmission pathways along the food chain of ESBL-producing *E. coli*, and the assessment of related risks for humans.

As part of the RESET project we develop a method to estimate the probable contribution of the different animal reservoirs to human infections with ESBL-producing *E. coli,* adapting a Bayesian source attribution model based on a microbial subtyping approach for Salmonella published by Tine Hald in 2004 (1).

Instead of using serotype information we define subtypes by incorporating information on ESBL-genes and their prevalences in animals and humans, determined in several studies within the RESET project.

We intend to give a short insight into the method of source attribution and want to present our first estimates on the contribution of different animal reservoirs to the German ESBL-producing *E. coli* prevalence in humans.

References:

(1) Hald, T., Vose, D., Wegener, H.C., Koupeev, T., 2004. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal 24, 255-269.

E 22

Investigations of the ecology of *Salmonella spp.* in fattening pig herds

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Keywords: Salmonella, pigs, cultural examination

The dissemination of Salmonella along the food chain is an important ongoing challenge within the scope of consumer protection and public health. However, most of the existing data have been collected selectively on the different stages of the pig production.
For this reason this study is supposed to contribute to integrated risk assessments.
The aim is to collect data across several stages within the pig production chain and the identification of possible risk factors for the entry of Salmonella into fattening pig herds and their spread within the herds.
Therefore, a longitudinal study in five conventional fattening farms is performed. These farms have been selected due to the results of the German serological Salmonella monitoring (sero-prevalence) and previous bacteriological detections.
Approximately, 1500 samples in total will be collected. Sampling includes the direct and indirect environment of the animals as well as pooled faeces samples from the pen floor.
Per farm, two groups from birth to finish will be repeatedly examined. Sampling will be carried out during weaning, growing and fattening periods. All samples will be culturally examined and isolates will be typed. Farm data are gathered using a questionnaire to identify possible risk factors. First results from the study will be presented on the congress.

E 23

Studies on the epidemiology of campylobacteriosis in Germany

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Keywords: Campylobacter, epidemiology, source attribution

In Germany, campylobacteriosis has been notifiable since 2001. In contrast to other gastrointestinal diseases, the annual number of reported cases has increased continually over the last years. With more than 70,000 reported cases in 2011, *Campylobacter* is now the most common cause of notifiable bacterial gastrointestinal diseases in Germany. Children <5 years and young adults are mainly affected. Most cases occur in the summer. Consumption of chicken meat but also other foods, contact to animals or contaminated water sources are possible risk factors for *Campylobacter* infections. A case-control-study was started in November 2011 with the aim to identify relevant risk factors for *Campylobacter* infections in Germany. Cases and healthy control persons in Berlin and Brandenburg are being interviewed regarding their exposure to possible risk factors, e.g. food items, using a standardised questionnaire. Furthermore, *Campylobacter* isolates from patients as well as food, animal and environmental isolates collected in the defined study area are being analysed by multilocus sequence typing. This interdisciplinary study will identify age-specific and season-related risk factors of campylobacteriosis and attribute types of human *Campylobacter* isolates to certain sources. Preliminary results of our study essential for the implementation of targeted control measures to reduce the number of *Campylobacter* infections will be presented.

E 24

Incidence of acute gastrointestinal illness among adults in Germany: a population-based survey

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Keywords: Infectious gastroenteritis, Disease burden, Risk factors

Population-based estimates of incidence and risk factors for acute gastrointestinal illness (AGI) are important for infectious diseases surveillance and health care planning. The majority of AGI is caused by infectious agents and a significant proportion is of foodborne origin.

We conducted a nationwide representative cross-sectional telephone survey among 21,262 adults over a 12-month period in 2008 and 2009. Participants were asked for having had either diarrhoea or vomiting in a 4-week-recall period. We excluded all subjects who had chronic gastrointestinal diseases from the analyses.

We estimated 0.87 episodes per person per year (95% confidence interval (CI): 0.82-0.92), corresponding to 59.4 million episodes of AGI annually in adults, which results in 22.5 million outpatient visits, 18.0 million hospital days and 58.1 million days of work lost. We observed an overall declining trend of AGI with increasing age. Diarrhoea was more often reported than vomiting. There is no difference in geographical distribution of AGI in Germany on district level. The mean duration of illness was 3.8 days and did not differ between age groups. Altogether 10.6% of all AGI cases have reported having taken an antibiotic therapy. Social factors seemed to be no or only weak predictors in comparison to state of health and health behaviour characteristics.

The impact of AGI in terms of social cost due to disease burden, health care utilisation and absence from work can be regarded as high.

E 25

In vivo studies into the persistence of *Toxoplasma gondii* in poultry

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Keywords: T. gondii, poultry, persistence

Toxoplasma (T.) gondii is a widely spread protozoon in humans, mammals and poultry forming tissue cysts in several organs. About the duration of tissue cyst persistence in poultry, nothing is known yet. The aim of the study was to investigate the persistence and distribution of *T. gondii* tissue cysts in different organs of experimentally infected turkeys and broiler chicken.

24 turkeys and 12 broiler chickens were infected intravenously with $1x10^6$ tachyzoites of the NED strain. Weekly, blood was drawn to determine the serotiter. Turkeys were slaughtered 4, 8, 12 and 16 weeks post infectionem (p.i.), and broiler chickens 5 and 10 weeks p.i., respectively (n = 6 per time). Organ samples from 16 different localizations were analyzed with a nested PCR based on the B1 gene for *T. gondii*.

All infected turkeys were tested seropositive for *T. gondii*-antibodies by kinetic ELISA. *T. gondii* DNA was detected in 45 of 384 organ samples (11.7%). In 20 samples of edible organs *T. gondii* DNA was found (5.2%). Six turkeys were tested PCR-negative in all organs. Positive samples were found at every time p.i. and there was no significant difference regarding the number of positive samples between the investigated points in time.

We could show that *T. gondii* in turkeys is persisting in different organs at least 16 weeks after an infection. This implies that an infection at an early time point after hatching might persist until the end of the conventional rearing period.

E 26

Serotyping of *Toxoplasma gondii* in cats (*Felis silvestris catus*) in Germany

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Keywords: Toxoplasma gondii; Cats; Serotyping

Cats are definitive hosts of *Toxoplasma gondii* and play an essential role in the epidemiology of this parasite. We investigated if cats are able to develop type-specific antibodies against different clonal types of *T. gondii* and examined the *T. gondii* types that are responsible for infection of cats as intermediate hosts for this parasite in Germany.

To this end, we developed a serotyping test based on a peptide microarray with 27 synthetic polymorphic peptides. Cat reference sera, i.e. sera of *T. gondii* type I- (n=17), type II- (n=3), type III- (n=1) infected, and of *T. gondii* seronegative cats (n=52) were used to establish the assay. For testing cats from Germany to determine their clonal type-specific antibody response, 86 sera from naturally infected *T. gondii*-seropositive cats were serotyped.

Based on the intensity and frequency, by which type-specific peptides were recognized, the assay correctly predicted the type of infection in reference cats. 94% (81/86) of naturally infected cats showed the strongest reactions with clonal type II-specific peptides and recognized similar peptide patterns as observed in the analysis of type II-infected reference cats (Supervised Kohonen Network analysis).

The results of our study show that cats mount a clonal type-specific antibody response against *T. gondii*. Serotyping of seropositive cat field sera from Germany revealed patterns resembling those observed after a clonal type II *T. gondii* infection.

E 27

Amblyomma variegatum ticks from Madagascar are highly infested with *Rickettsia africae* and could be vectors for human spotted fever rickettsioses

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Keywords: tick-borne infections, spotted fever group rickettsiosis, Madagascar

Hard ticks are potential vectors for the transmission of bacterial infections to humans. The most important human tick-transmitted disease on the African continent is spotted fever group rickettsiosis. Nothing is known so far about the role of ticks as reservoirs and vectors for zoonoses on the island Madagascar.
We collected ticks from cattle in a central slaughterhouse in the capital Antananarivo. Ticks were identified as *Boophilus microplus* and *Amblyomma variegatum,* which is a known vector of rickettsiosis in humans on the African continent. We therefore investigated *Amblyomma* ticks for the presence of rickettsial DNA, using a genus-specific real time PCR targeting conserved regions of the ompB gene. Of 84 *Amblyomma* ticks tested, 62 (73,4%) were positive for rickettsiae. OmpB sequencing showed a high degree of conservation and a high homology to *R. africae* detected in ticks from Ghana and Cameroon.
Nearly 1000 human sera were collected from healthy pregnant women in Madagascan villages and were analysed for the presence of antibodies against *R. conorii* as a surrogate antigen for spotted fever group rickettsioses. The seroprevalence was about 1%.
Amblyomma variegatum ticks from different regions of Madagascar are highly infested with *R. africae*. Tick contact may be a risk factor for spotted fever group rickettsioses in Madagascar. The presence of antibodies against *R. conorii* in humans shows that rickettsial infections are indeed occurring in Madagascar, but the rate of human infections seems to be lower than may be anticipated from the high rate of infested ticks.

E 28

MLST for genotyping of *Vibrio parahaemolyticus*

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Keywords: Vibrio parahaemolyticus, MLST

The ubiquitous bacteria *Vibrio* (*V.*) *parahaemolyticus* is found naturally in marine and estuarine waters, including aqua- and mariculture. It is one of the leading causes of seafood-borne bacterial illnesses. The global distribution of potentially pathogenic *V. parahaemolyticus* and its genetic relations can be analyzed by Multilocus Sequence Typing (MLST). Based upon the comparison of nucleotide sequences of defined internal fragments of seven reference genes, UPGMA-Analysis was performed and Minimum Spanning Trees were compiled.

In this study 78 *V. parahaemolyticus* isolates of different geographic origin (e.g. Asia, Europe and South-America) and matrices (bivalves, shrimp and seawater) were applied to MLST. After dividing the original *gyr*B and *rec*A fragments into two parts, the allelic profile of 69 isolates could be completed. In total, 65 new alleles were found (7 *dna*E, 15 *gyr*B, 9 *rec*A, 9 *dtd*S, 6 *pnt*A, 10 *pyr*C and 9 *tna*A). 28 new Sequence Types (ST) consisting of combinations of new and already published alleles were identified. The majority of the analyzed *V. parahaemolyticus* (n= 46) strains originated from shrimp of three different regions of Sri Lanka. They were assigned to 15 different STs. Ten of this 15 STs were specific for one region, two STs were found in two and three were present in all regions. Additionally, isolates from food samples (n=24) and two clinical reference strains were typed. They belonged to 23 different STs. The STs of *V. parahaemolyticus* strains were independent concerning their geographic origin or matrix.

In summary, *V. parahaemolyticus* strains investigated, could be assigned to multiple already published STs or new STs. This reflects a high genetic diversity. Neither a regional (Sri Lanka) or a global dominating ST was identified. Analysis of the MLST data via eBURST showed a high proximity between environmental and clinical strains.

E 29

Establishment of a *Giardia duodenalis* database and biobank for functional epidemiology

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Keywords: Giardia duodenalis, gastrointestinal infection, functional epidemiology

Giardiasis is a highly prevalent gastrointestinal infection caused by the flagellate parasite *Giardia duodenalis*. Humans can be infected by two genotypes (assemblage A and B) that also are potentially zoonotic. In Germany, giardiasis is a reportable disease with 4-6 reported cases/100,000 that are accompanied by a broad spectrum of gastrointestinal symptoms. Chronic or relapsing courses of the disease are relatively common, and a considerable number of treatment failures upon standard therapy (e.g., metronidazole) is reported by clinicians. It is currently unknown, which specific virulence factors and/or host risk factors contribute to the pathophysiology of giardiasis, mainly due to the lack of functional epidemiological studies.

In this project we aim at establishing a database-linked biobank collection of *G. duodenalis* isolates from humans and animals to combine studies on molecular epidemiology with functional testing of a respective isolate and thereby to identify e.g., virulence factors.

As introduced last year, we are in an ongoing process of sample collection, characterization, and cultivation of Giardia isolates from humans and animals. Here, an update of the present status of the project will be presented.

E 30

Antimicrobial resistance and intraspecies variability of indicator bacteria

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Keywords: antimicrobial resistance, indicator species, Ecoff-values

The Bavarian Monitoring of Antimicrobial Resistance Program (BAVMAP) provides well comparable resistance data from the sources “man”, “animal” and “food” for *E. coli* (n = 3036), *Ent. faecalis* (n = 2729), *Ent. faecium* (n = 1015), and other indicator species. The highest rates of resistant bacteria were found in pigs and in human stationary patients. However, *E. coli* – in contrast to other *Enterobacteriaceae* – were most frequently resistant when isolated from cattle. Universally susceptible against 22 suitable antibiotics were only 4.4 % of porcine *Ent. faecalis*, but 24.2 % of human and 36.0 % of bovine *Ent. faecalis*. By contrast, 78.3 % of porcine *Ent. faecium*, 42.6 % of bovine *Ent. faecium* and 11.8 % of human *Ent. faecium* were universally susceptible. The mean wildtype-MICs for one species differed, depending on the source. In order to highlight why species from identical ecological niches differ basically in their antimicrobial resistance, isolates were investigated for their phylogenetic offspring by Clermont-phylotyping, ERIC-PCR, and RAPD-PCR. Human, porcine and bovine bacterial populations of identical species were dominated by different phylogenetic lineages. This might considerably modulate the selection of antibiotic resistance and might also cause a need to shift the epidemiological cut-off-values. Thus, intraspecies-variability should be borne in mind when choosing adequate indicator species, which is essential for the informative value of each monitoring program.

Poster Session Innate and adaptive immune response

I 01

Identification of Interferon stimulated genes in the chicken

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As in mammals type I interferons (IFN) are an essential part of the antiviral immune response in the chicken. But while in mice Mx proteins are the key effector molecules during the immune response against influenza viruses, the chicken's Mx protein is induced by IFN but does not mediate antiviral activity. Hence the aim of this study was to identify chicken Interferon stimulated genes (ISGs).
Therefore we combined an extensive comparative database analysis to identify chicken homologues to mammalian ISGs and a transcriptome analysis of spleen and lung at different time points after i.v. injection of recombinant IFN. Array analysis detected for both tissues at all time points massive changes in the transcriptome. Since only 25% of the regulated genes in the lung and even less of the regulated genes in the spleen were in agreement with the chicken ISGs from databases, this *in vivo* experiment led to the identification of a multitude of so far unknown chicken ISGs.
To test these results in different infection models, further microarray studies were performed on lungs and spleens from chicken infected with New Castle Disease (NDV) or FluAV (H5N1/R65). These investigations confirmed a large part of the newly identified ISGs. In all experiments a rapid and massive induction of IL-22 and IL-6 was detectable as well as a strong upregulation of the chemokines CCL19 and K203 (a chicken MIP family member). Another highly induced gene was the chicken homologue for IFIT-5, suggesting a conserved antiviral activity of the IFIT gene familiy.

I 02

Humoral and cellular adaptive immune reactions of raccoons against rabies

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Keywords: rabies, raccoon, immune response

Rabies virus (RABV) causes an inevitably fatal disease in mammals once clinical signs develop. Raccoons carry a distinct variant of RABV and are major reservoirs of rabies virus in USA. Since two populations established in late 40ies raccoons become more important as possible vectors of rabies in Germany, too. However, limited information is available about resistance or immunity against rabies and possible risks of transmission of European virus strains by local raccoon populations. One reason is the lack of genome sequence information, of molecular markers of immune cells and of molecular tools to investigate the immune system and immune responses of raccoons to viral pathogens.

To get first insights into the immune system of raccoons we will identify humoral and cellular factors of the immune system, develop molecular tools for serological assays and real time PCR based analysis and use these tools to determine the humoral and cellular immune response of experimentally infected raccoons.

To establish serological assays raccoon immunoglobulins were purified and used to produce anti-raccoon Ig-antibodies. The transcriptome of raccoon leukocytes will be analysed by deep sequencing. Characterized leukocyte surface markers and immunoregulatory molecules will serve as molecular tools. Both approaches will be combined to investigate the interaction between Rabies virus and raccoon immune system during infection and vaccination in *in vitro* and *in vivo* experiments.

I 03

Humoral and cellular adaptive immune reactions of European bats against rabies viruses

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Keywords: bat, rabies virus, immune response

Bats combine several features making them to an important reservoir of zoonotic viruses:a) These mammals can fly and migrate to different areas in the world; b) They evolved about 52 million years ago and c) they are one of the most abundant, diverse and widely distributed vertebrate family. Bats are identified to host more than 85 viruses and several are associated with animal and human epidemics. As the natural hosts of rabies virus and related lyssaviruses, bats have become one focus for the control of rabies not only in South America and Africa, but also in Europe and North America. In contrast to this, the knowledge about pathological and immunological processes during viral infections is very limited.
Here we show the general approach to analyse the humoral and cellular immune response of European bats to rabies virus and the first results in this project. To analyze the humoral immune responses against rabies virus and to determine the biological significance of different immunoglobulins, IgM and IgG are isolated from sera and used to generate poly- and monoclonal antibodies. To investigate the IgG isotypes, cellular receptors and immunoregulatory factors, 454-deep sequencing technique is used to sequence the transcriptome of different lymphoid organs. The obtained genetic information and developed molecular tools will be used for the analysis of immunological processes in bats in response to rabies virus to optimize strategies for diagnosis and disease control.

I 04

The papain-like protease domain of a European SARS-related bat coronavirus inhibits interferon induction and promotes viral growth in human cells

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Keywords: SARS-related bat coronavirus, Papain-like protease, Interferon antagonist

Bats harbour a high diversity of coronaviruses (CoV) and are assumed to be the reservoir host for severe acute respiratory syndrome CoV (SARS-CoV). Intracellular countermeasures against viral infections like the Interferon (IFN) response can be antagonized by viral proteins. The SARS-CoV papain-like protease domain of nonstructural protein 3 (N3plp) targets the interferon regulatory factor 3 (IRF3) and thus interrupts the IFN induction pathway.
In the presented study we investigated if the homologous N3plp domain from a European SARS-related bat-CoV (bN3plp) is functional as an IFN antagonist in bat and primate cell cultures.
In order to analyze if bN3plp inhibits the IFN induction pathway an IRF3 nuclear translocation assay was performed in primate kidney cells (MA104). Both overexpressed N3plp domains blocked the translocation of a cotransfected IRF3-GFP at similar levels. Moreover, both proteases showed an inhibition of IFN-beta promoter activation in primate and bat cell lines.
Recombinant chimeric SARS-CoVs containing heterologous bN3plp core domain from the bat virus or the combination of bat core and transmembrane domains were generated by reverse genetics. Compared to wild type virus both chimeric viruses grew with only a slight delay and to high titers in human cells, indicating a functional compensation by bN3plp.
In summary these data suggest that bN3plp is able to antagonize the type I IFN response in primate and bat cells without further adaptation.

I 05

The influenza nucleoprotein-specific memory B-cell pool is composed of multiple clonotypes with little clonal hypermutation

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Purpose/Objective: Immunization against influenza virus induces a pauciclonal expansion of antigen-specific B-cells. The resulting clonal plasma cell pools contain a highly diverse spectrum of somatically mutated cells. To examine how the antigen-specific B-cell diversity is maintained, we examined the repertoire of influenza nucleoprotein (NP)-specific memory B-cells two weeks after vaccination.
Material and Methods: Influenza NP-specific memory B-cells were isolated in a multi-step process from peripheral blood using protein-coated magnetic beads. The purity of the NP-specific B-cells was determined by ELISpot. Immunoglobulin G variable regions were amplified from single B-cells by RT-PCR, sequenced, cloned and expressed as recombinant monoclonal antibodies. Antigen specificity was determined by ELISA.
Results: Influenza NP-specific B-cell preparations were highly pure. More than 60 different clonotypes belonging to immunoglobulin heavy chain variable subgroups 1, 3, 4, 5, and 7 were identified in 3 subjects. The clonotype repertoire varied significantly between the subjects. Somatically mutated variants were detected for a minority of the clonotypes. Several memory B-cells had low numbers of somatic mutations.
Conclusions: Large numbers of different clonotypes and few somatically mutated variants indicate that the memory B-cell repertoire is primarily composed of different clonotypes rather than somatically mutated variants. Additionally, despite probable multiple exposures to influenza virus the vaccination induced several new influenza nucleoprotein-specific memory B-cell clonotypes in all donors.

I 06

Influenza B virus NS1 protein controls riplet-mediated RIG-I activation

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Keywords: RIG-I, Type I Interferon, ubiquitination

The retinoic acid inducible gene I product (RIG-I) functions as a cellular sensor detecting viral 5´-PPP-RNA during influenza virus infection, triggering the antiviral I interferon response. RIG-I activity is tightly controlled by ubiquitination, mediated by at least three ubiquitin ligases. Previously, it was shown that the influenza A virus NS1 protein inhibits RIG-I activation by interfering with its TRIM25-mediated ubiquitination.

Here, we investigated how the highly divergent influenza B virus (FLUBV) blocks the antiviral response. Using RNAi we show that RIG-I is essential for FLUBV-induced IFNβ production. Reporter assays of transfected cells revealed that the E3 ligase Riplet has a very strong impact on RIG-I induced IFNβ promoter activity and enhanced RIG-I activation by influenza virus RNA. Riplet stimulates covalent ubiquitination of RIG-I. Consistently, mutation of key lysine residues in RIG-I abrogated its activation by viral RNA or Riplet. The FLUBV NS1 protein (B/NS1) interacts with RIG-I and Riplet in bimolecular fluorescence complementation assays. B/NS1 inhibits the RIG-I-dependent induction of the IFNß promoter both in infected and transfected cells. The IFN-inhibitory function was mapped to the C-terminal region of B/NS1.

In conclusion, our data suggest that B/NS1 inhibits the RIG-I dependent signalling pathway by binding to Riplet acting upstream of RIG-I and thus executing an important pathogen-host interaction in the influenza virus replication cycle.

I 07

Susceptibility of *Mycobacterium avium subsp. paratuberculosis* towards human, mouse and bovine enteric antimicrobial peptides

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Keywords: innate immunology, mucosal immunology, enteric antimicrobial peptides

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne´s disease, a chronic degenerative wasting disease that primarily infects ruminants. In cattles, transmission primarily takes place from the mother to the newborn, whereas adult individuals seem to be rather resistant to primary MAP infection. Paratuberculosis is transmitted via the oral route, indicating that the intestinal mucosa is the port of entry. The bovine repertoire of antimicrobial peptides, endogenous cationic peptides with broad antibacterial, antiviral and antiprotozoon activity, consists primarily on a large family of cathelicidins. MAP infection has also been linked to human cases of Crohn's disease, a chronic inflammatory disease of the intestinal tract. Important antimicrobial peptides produced by Paneth cells in the small intestinal crypts are human defensin (HD)5 and 6. Impaired Paneth cell development and reduced defensin expression was described in patients with Crohn's disease. Our own results demonstrate an enhanced susceptibility of the gut epithelium in neonatal *versus* adult mice for MAP infection. Whereas the cathelicidin Cramp is expressed in the neonatal gut mucosa, adult mice exhibit expression of a large family alpha-defensins (cryptdins). We therefore analyzed the susceptibility of MAP towards a number of bovine, human and mouse antimicrobial peptides in order to evaluate their potential contribution to the host susceptibility displayed by MAP.

I 08

Reduced cytokine expression in *Mycobacterium avium* ssp. *paratuberculosis* infected macrophages results from the absence of calcium release from intracellular stores

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Keywords: Mycobacterium avium spp. paratuberculosis, IL-6, calcium

We have recently shown that pathogenic mycobacteria such as *Mycobacterium avium* ssp. *paratuberculosis* (MAP) – in contrast to non-pathogenic mycobacteria and LPS – induce a restricted activation of macrophages resulting in a low inflammatory response. One of the key findings was the absence of interleukin-6 (IL-6) in the supernatants of MAP-infected macrophages. In this study we identified signaling pathways in MAP-infected RAW264.7 macrophage-like cells responsible for this IL-6 suppression by comparing MAP-infected cells with LPS-stimulated macrophages. qRT-PCR analyses revealed a significantly lower mRNA level of IL-6 and TNF in MAP-infected macrophages. Although the IL-6 gene was transcriptionally activated, promoter analysis using transient expression of a luciferase reporter driven by the IL-6 promoter resulted in promoter activity in both LPS and MAP-stimulated cells. Experiments employing pharmacological inhibitors of signaling pathways involved in IL-6 expression demonstrated that the release of Ca^{2+} from intracellular stores is essential for LPS-induced IL-6 expression. *Vice versa* treatment of MAP-infected cells with the calcium ionophore A23187 to increase $[Ca^{2+}]_i$ induced IL-6 expression. Together our results provide evidence that inhibition of Ca^{2+} release from intracellular stores, most probably by affecting chromatin remodelling, is responsible for the suppression of IL-6 in MAP-infected macrophages.

I 09

Uptake and intracellular processing of *Chlamydia* by antigen presenting cells

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Keywords: Antigen presenting cells, Autophagy, Cross-presentation

Professional antigen presenting cells (APCs) play a central role in the maintenance of the immune system. Dendritic cells (DCs) are likely to be the first APCs that get into contact with chlamydial antigens and thus are of crucial importance for initiating respective immune responses. However, DC-mediated uptake and intracellular processing of chlamydia are not entirely understood, which is also true for the intracellular transport involved in MHC I-presentation of chlamydial antigens.

We are interested in the involvement of C-type lectin receptors (CLRs) in the DC-mediated uptake of Chlamydia. Therefore, we have used the mouse DC cell line JAWS II, which expresses detectable amounts of different antigen uptake receptors. We observed significant changes in the expression as well as in the intracellular localization of C-type lectins during chlamydial infection. Most interestingly, our studies on DCs also suggest that autophagy seems to represent an important route by which chlamydial antigens are delivered to MHC I-cross-presentation. Thus, co-localization experiments with infected DCs show a strong signal overlap between different autophagy markers and chlamydial structures.

Taken together, the results of our work will allow us to follow the route of chlamydial antigens in infected DCs from their uptake and intracellular processing right up to their MHC I-surface presentation to cytotoxic T-cells.

I 10

Replication of different *Coxiella burnetii* (*C. b.*) strains in macrophages of ruminants and induced host cell response

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Keywords: Coxiella burnetii, Macrophages

Although domestic ruminants are the main source of human Q fever, little is known about the immunobiology of the obligate intracellular bacterium *C. b.* in animals. As macrophages are considered to be the natural target cells, monocyte derived macrophages (MDM) from cattle, sheep and goats were inoculated *in vitro* with either of five *C. b.* strains differing in origin and genotype. *C. b* replication rates were calculated by determining the median tissue culture infective dose at different time points after inoculation in an endpoint titration method. The MDM reaction to inoculation was characterized by quantifying cytokine expression (IL-1 β, IL-6, IL-8, IL-10, INF-γ, TNF-α, TGF-β, MCP1, RANTES, iNOS) via RT-PCR. *C. b.* replicated in MDM with strain- and species-specific kinetics. In bovine MDM *C. b.* numbers of strains especially Scurry increased by 2 to 3 orders of magnitude 14 d p. i.. In contrast, *C. b.* did not significantly replicate in ovine and caprine MDM, yet retained viability for 7 days. MDM differentially responded to inoculation within 3 h p. i.. Responses were dominated by upregulation of pro-inflammatory and TH1-related cytokines (IL-1 β, IL-12). However, *C. b.* inoculation also stimulated expression of IL-10 and IL-8. These findings provide the first detailed insight into *C. b.*-macrophage interactions in ruminants at cellular level and may serve as a basis for assessing the virulence and the likelihood of animal-to-human transmission of *C. b.* strains.

I 11

Serodiagnostic and seroprevalence of campylobacteriosis and post-*Campylobacter*-sequelae

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Keywords: Campylobacter, Serodiagnostic, Guillain-Barré-Syndrome

Due to molecular mimicry of the campylobacterial LOS and gangliosides of the human body, post-enteritic complications such as reactive arthritis (RA), inflammatory bowel disease (IFB) and Guillain-Barré-Syndrome (GBS) may arise. According to previous studies, up to 80% of GBS-cases were associated with campylobacteriosis.
An ELISA using the *C. jejuni* proteins P39 and OMP18 was prepared. 91 sera from GBS patients, 50 sera from RA patients and 39 sera from IFB patients were tested for specific IgA and IgG. Sera from 60 patients with acute campylobacteriosis and from 80 healthy blood donors served as control. ROC curves indicate that we could reach the optimal sensitivity/specificity ratio (≈0.86) using the P39 antigen alone. Combination of P39 with OMP18 or other antigens (incl. the whole cell lysate) results in a significant decrease of specificity that is not compensated by increased sensitivity. Using the P39-based ELISA we detected a *Campylobacter* seroprevalence (IgG) of 9% in healthy blood donors, 37% in GBS-patients, 52% in acute enteritis patients (79% seroprevalence including IgA and borderline positive), 54% in RA-patients and 44% in IBD-patients.
Thus, the proportion of post-*Campylobacter* GBS was largely overestimated and should be corrected downward to about one third of GBS-cases. Furthermore we present valid data for the proportion of post-*Campylobacter* RA and substantiate the discussion about the role of recurrent acute triggering IBD with serological data.

I 12

K203: an effector molecule in chicken immune defence against *Salmonella* infections

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Keywords: Immunology, Salmonella

Food-borne zoonotic infections of humans are still a major problem in Europe. In the case of human *Salmonella*-Infections over 40.000 registered infections are reported every year in Germany , few of them with a fatal outcome.
S. typhimurium and *S. enteritidis* are the two most important zoonotic serovars and responsible for over 75% of the human infections. Chicken are asymptomatic carriers of the pathogen, only day-old chicks may develop a systemic infection when infected with a high doses.
The control of infections in birds is an important strategy to prevent human infections. Therefore, a comprehensive understanding of the immune response of chicken to *Salmonella enterica* is crucial to develop new control strategies. In order to better understand the innate response of birds to infection, we performed global gene expression analysis using the micro-array technology, which let to the identification of candidate genes supposed to play an important role in the early immune defence of chicken against *Salmonella enterica* infections.
The CC-chemokine K203, which is supposed to be an ortholog of human CCL16, is the most strongly induced gene in Salmonella infected macrophages. However, its function is entirely unclear. Quantitative RT-PCR studies revealed that K203 is strongly induced in primary macrophages upon stimulation with particular TLR agonists (TLR3, 7 and 21) and IFN-γ. This gene is also induced in Salmonella infected gut tissue.
In order to study its biological role we have now cloned and expressed the K203 gene under different conditions. Flag-taged recombinant protein was purified and used to raise a polyclonal antiserum in rabbits. With these tools we now set out to investigate its chemotactic potential, to identify the cell type attracted and to study its *in vivo* role during bacterial infection.

Poster Session Pathogen-cell interaction

P 01

Human intestinal Caco-2 cells are susceptible to Tick-borne Encephalitis Virus infection

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Keywords: TBE virus, intestinal cells, alimentary route of infection

Tick-borne encephalitis virus (TBEV) belongs to a member of the genus flavivirus, family Flaviviridae. The three main subtypes are the European, Siberian and Far-eastern subtypes. Although the transmission of TBEV occurs mainly via a tick bite, recently a few TBE outbreaks caused by consumption of non-pasteurised milk and cheese products were reported. But until now, little is known about the alimentary route of TBE infection as well as the replication and persistence of this virus in human intestinal cells.

In this study, human intestinal Caco-2 cells were infected with the three subtypes of TBEV. RT-qPCR and plaque assay were used to monitor the kinetics of TBEV replication, respectively. The three strains of TBEV produced high viral copies and titers during infection. Transmission electron microscopy was used to show morphologic changes of Caco-2 cells infected with TBEV. In addition, the disruption of the cellular cytoskeleton with cytochalasin D and nocodazole leads to significant reduction in virus infectivity. Furthermore, cells treated with ly294002, a PI3K-specific inhibitor were highly resistant to infection, suggesting that virus replication may be dependent on the activation of PI3K/Akt pathway.

In conclusion, our results show that the human intestinal Caco-2 cells were sensitive to infection with three different TBEV strains. Moreover, we found that the biochemical inhibitor cytochalasin D, nocodazole, or ly294002, dramatically decreases TBE virus replication.

P 02

Establishment of *in vitro* methods to study the entry of neurotropic viruses into the central nervous system (CNS) via the olfactory pathway

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Keywords: cell culture, organotypic culture, olfactory epithelium

Rodent models usually represent the method of choice to study the pathogenicity of viruses. Neurotropic viruses often enter the CNS via the olfactory pathway employing initial infection of the olfactory epithelium (OE). This is known e.g. for vesicular stomatitis virus, herpes simplex virus, rabies virus, influenza virus and Borna disease virus (BDV). To reduce animal experiments and to enable studying viral entry mechanisms 2 different in-vitro-methods have been established and BDV has been used as model infection.

Dissociative cell cultures and organotypic cultures were prepared from rat OE and compared regarding their feasibility and practicability. The advantage of dissociative cultures is the possibility to study the impact of defined parameters. Co-cultivation with different types of cells is possible and neural growth can be assisted by culture supplements. The organotypic cultures enable research in an intact cellular network closely mimicking the natural situation. First results show that BDV could be detected successfully by immunofluorescence *in vitro*.

Both cultures have been proofed useful to study olfactory viral entry. Since material of different species can be easily taken by biopsy or during necropsy, these culture systems will also allow investigations of species barriers at the level of viral entry. This represents an important feature in case of zoonotic and emerging virus infections.

P 03

Analysis of host cell entry of severe fever with thrombocytopenia syndrome virus (SFTSV), a novel hemorrhagic bunyavirus

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Keywords: SFTSV Bunyavirus, entry, lectins

Infection by severe fever with thrombocytopenia syndrome virus (SFTSV), a novel hemorrhagic bunyavirus, induces severe illness with case-fatality rates of approximately 10%. In order to understand the viral pathogenesis, it is essential to identify which cells are targeted by the virus. The G-protein of bunyaviruses is the only viral surface protein and mediates host cell entry. We employed retro- and rhabdoviral vectors pseudotyped with the SFTSV G-protein to analyze cellular entry of SFTSV.
SFTSV-G pseudotypes were infectious for a broad range of human and animal cell lines, including retinal, lung, kidney and glioblastoma cells. Notably, pseudotypes bearing SFTSV- and Rift Valley Fever Virus (RVFV) G-proteins showed a similar cell tropism, while pseudotypes harboring LaCrosse Virus (LACV)-G infected all cell lines tested. Inhibition of endosomal acidification impaired SFTSV-G mediated entry, indicating that virus-cell fusion driven by SFTSV-G is triggered by low pH. The lectin DC-SIGN facilitates host cell entry of Phleboviruses. Therefore, we analyzed the role of lectins in SFTSV entry. DC-SIGN, DC-SIGNR and Lsectin enhanced cellular entry of pseudotypes bearing SFTSV and RVFV G-protein, whereas infection by LACV-G pseudotypes was not augmented, indicating that lectin engagement is not universal among bunyaviruses. In sum, our results show that pseudotyping is a suitable technique to study SFTSV entry and suggest similarities between SFTSV and RVFV entry.

P 04

Proteolysis of ACE2, the SARS-coronavirus receptor, by type II transmembrane serine proteases: Identification of the cleavage site and importance for SARS-spike-driven host cell entry

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Keywords: ACE2, SARS-CoV, TTSPs

The SARS-coronavirus (SARS-CoV) employs angiotensin-converting enzyme 2 (ACE2) as receptor for entry into host cells. We and others have previously shown that TMPRSS2 and HAT, members of the type II transmembrane serine protease (TTSP) family, activate the spike (S) protein of SARS-CoV. In addition, it was reported that TMPRSS2 cleaves ACE2 and thereby increases SARS-S-driven cellular entry. We investigated the TTSP cleavage site in ACE2 and its importance for SARS-S-dependent cellular entry. Coexpression of ACE2 with TMPRSS2, HAT and several other TTSPs resulted in the production of short, identical C-terminal cleavage fragments, indicating that several TTSPs facilitate ACE2 proteolysis at a single membrane proximal site. Analysis of cleavage fragments by mass spectrometry identified a putative cleavage site and initial analysis of ACE2 mutants supported an important role of these amino acids in ACE2 proteolysis. Finally, evidence was obtained that expression of TTSPs on target cells can augment SARS-S-driven entry while proteases with mutated catalytic center are inactive, suggesting that TTSP cleavage of ACE2 might augment viral infectivity. A comprehensive analysis of the cleavage site and its importance for SARS-S-driven cell-cell and virus-cell fusion will be presented.

P 05

Identification of ganglioside receptors of influenza A virus hemagglutinins and EHEC subtilase cytotoxin (SubAB)

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Keywords: glycolipids, cell surface receptors, protein-carbohydrate interaction

Sialic acid-mediated cellular interaction of influenza A viruses and some toxins from zoonotic pathogens represents the initial step in the infection process. We investigated gangliosides (sialylated glycolipids) of human and animal origin for their receptor potential of porcine H1N1 and avian H5N1 influenza A virus strains, recombinant soluble hemagglutinins H7 and H9 derived from avian influenza viruses and the recombinantly produced B-subunit of the subtilase cytotoxin (SubAB) from enterohemorrhagic *E. coli* (EHEC). Porcine H1N1 and avian H5N1 influenza A viruses exhibited a distinct binding pattern towards individual gangliosides in comparison to human H3N2 influenza A viruses. In-depth structural analysis of receptor gangliosides by mass spectrometry revealed data on the monosaccharide sequence as well as on the terminally linked sialic acid (α2-3 *versus* α2-6), the prevalent motif of ganglioside-binding proteins. The two soluble H7 and H9 subtypes differed in the spectrum of recognized gangliosides, whereby H7 showed a clear preference to α2-3-sialylated gangliosides. Binding assays of the EHEC-derived B-subunit of SubAB revealed preferential binding towards gangliosides from animal sources and rather low interaction to gangliosides of human origin. The question whether lung and intestinal epithelial cells do express influenza virus- and SubB-binding gangliosides, respectively, has to be addressed in further investigations.

P 06

Using quantitative proteomic analysis of recombinant influenza A virus to define the interactome of the viral NS1 protein

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Keywords: influenza A virus, NS1, interactome

The influenza A virus NS1 protein modulates various cellular processes including the antiviral interferon response, cellular RNA metabolism and phosphoinositide 3-kinase signalling. NS1 has a modular structure consisting of an N-terminal RNA binding and dimerization domain (aa 1-73) and a C-terminal effector domain (aa 74-231). We wish to characterize the pleiotropic modes of action for the viral NS1 protein by defining the whole ensemble of cellular binding partners in the infected host cell using quantitative proteomic methods.

Towards this goal we employed a recombinant influenza A virus expressing a fusion protein including NS1 amino acids 1-104 and the autofluorescent GFP protein combined with stable isotope labeling by amino acids in cell culture (SILAC). Lysates of infected cells were subjected to immunoprecipitation through the GFP trap method followed by detection of bound proteins using high resolution mass spectrometry by coupled LC-MS/MS. This resulted in the identification of a distinct set of high confidence cellular factors. The list contained proteins such as PABP1 and hnRNP-F that have previously been described to bind to the NS1 protein. In addition, several novel NS1 interactor candidates were found. We will present an analysis of novel NS1 binding proteins and will discuss the potency of novel proteomic tools to define host virus interactions.

P 07

Specific amino acid in NS1 of highly pathogenic avian influenza A viruses effect the viral replication in mammalian cell.

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Keywords: Influenza virus, host range

In mammalian cell culture systems the FPV-GD-NS reassortant shows increased replication ability including alteration of the polymerase activity compared to the wt FPV, while the FPV-Ma-NS is strongly impaired. Nevertheless, in avian cell culture systems both reassortant viruses as well as the wild type FPV replicate similar. The NS-genes of GD and Ma only differ in 8 amino acids (aa) in the NS1- and 2 aa in the NS2/NEP protein. Consequently, we are analyzing which aa or combination of aa in the GD-NS1 would be responsible for the enhanced replication of the FPV-GD-NS virus in mammalian cells. Therefore, we employ mutational analysis and generation of recombinant reassortant viruses with Ma-NS segments altered towards the GD-NS sequence to examine several aspects of viral replication, such as (i) viral propagation/growth rate and transmission/host range, (ii) an effect on the viral replication, transcription and the export of viral genome and (iii) effects of the altered NS1 proteins on the innate immunity and cell-mediated apoptosis. The analysis of recombinant reassortants in mammalian and avian cell culture systems shows that specific changes in the aa-sequence of the Ma-NS segment indeed increase the replication ability of the recombinant reassortant virus in mammalian cell culture, but interestingly none of the changes increased the replication of the different recombinant reassortants in avian cell culture. The results so far indicate no selection advantage of the NS1 sequence differences in the FPV-GD-NS virus in avian cells and probably they will therefore not be selected, even though in mammalians systems they might be advantageous.

P 08

The NS1 protein of seasonal influenza A viruses inhibits virus- and IFN-dependent induction of ISG15 in human cells

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Keywords: Influenza A, ISG15, species specificity

Influenza A virus (IAV) infection provokes an antiviral immune response including activation of IFN and IFN-stimulated proteins. One of these proteins is ISG15, which is a ubiquitin-like polypeptide that can be covalently attached to target proteins. The impact of ISG15 on IAV propagation as well as on species specificity is subject of our investigation.
Our initial findings revealed a strong induction of ISG15 in A549 cell cultures upon infection with seasonal IAV. We observed on single cell level that this induction occurred predominantly in uninfected cells, whereas little ISG15 was detected in the initially infected cells. A population of both infected and ISG15-positive cells was present after infection with a ΔNS1 mutant virus indicating a role for NS1 in suppressing ISG15 in the infected cells. This increased double-positive population was also observed upon infections with low-pathogenic avian viruses (LPAIV). Transfection-based experiments demonstrated that the presence of an active viral minigenome was sufficient to induce ISG15, which was inhibited by co-expressed NS1 protein. Transfection of NS1 proteins of seasonal IAV remarkably reduced IFN-stimulated ISG15-induction. Knockdown of ISG15 had no effect on replication of seasonal IAV but led to enhanced replication efficiency of two LPAIV.
Our results indicate that the NS1 protein of human IAV suppresses not only virus- but also IFN-dependent ISG15-induction and imply a role of ISG15 in limiting IAV host range.

P 09

A siRNA screen to detect influenza virus strain-specific differences in required host cell factors

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Keywords: influenza, screen, strain-specific

Influenza viruses (IV) depend on the host cellular machinery to complete their life cycle. Thus, host factors might be attractive targets for antiviral compounds. To identify cellular genes required for IV infection, six genome-wide screens have been performed by independent laboratories, and 1449 human genes have in total been proposed as host dependency factors for IV replication. The strains applied in these studies had in common that they originated from seasonal human influenza viruses of genus A. Thus, it is unclear if the published factors are also required for influenza virus strains with a genotype and phenotype differing from that of the previously employed isolates. This information is, however, essential for the development of a broadly active drug. Therefore, we will perform a short interfering RNA (siRNA) screen using six IV strains differing in their genetic and phenotypic properties (Spanish influenza, H5N1 avian influenza, swine-origin influenza, influenza B, contemporary seasonal influenza A, A/WSN/33). The genes to be screened comprise 352 published host dependency factors as well as the human kinome (719 genes). This study will help to unravel IV strain-specific differences in the reliance on host cell genes and may reveal novel options for antiviral therapy.

P 10

Species dependent host factors of influenza A virus replication

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Keywords: Influenza A Virus, host tropism, RNAi screen

The Influenza A Virus (IAV) is one of the most influential pathogens causing annual epidemics, and in rare circumstances, global pandemics. Current strategies involved in combating the virus are not always successful, thereby new strategies to prevent the spread of resistant IAVs are required. One possibility is the host-directed therapy, which targets host cell factors essential for IAV replication. In this regard, four genome wide RNAi-based screens have been performed yielding several human host cell factors essential for the virus to complete its life cycle. However, it still remains unclear, if the identified host cell factors are relevant in other species. IAVs are capable of infecting a wide range of animal hosts but only the natural reservoir, aquatic birds, can maintain some viral strains, which could result in serious pandemics when transmitted to other hosts. Importantly, the host cell environment strongly determines the ability of a given IAV strain to infect a particular cell, thereby decreasing the possibility of interspecies transmission. We now intend to identify additional similarities and differences between the human, the avian and the porcine system, often called the mixing vessel as it supports transmission of avian IAVs to humans. To assess it a targeted RNAi screen in avian and porcine cells, shall be performed. This will lead to a better understanding on the viral tropism and provide new target candidates for drug development.

P 11

A pandemic H1N1 (pH1N1) G222 hemagglutinin (HA) genotype is not necessarily more pathogenic in mice than a D222 or E222 variant

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Keywords: pandemic H1N1, mouse model, pathogenicity

Beside mild courses, pH1N1 can also lead to severe or fatal disease in humans. It was suggested that the D222G mutation in HA enhances the binding capacity of pH1N1 to α2,3-linked sialic acid receptors (α2,3SA) and is associated with severe lung disease in humans and in mice due to this receptor adaptation.
These results prompted us to compare receptor binding properties and pathogenicity of three pH1N1 isolates with polymorphism at HA position 222 in mice. D222 and E222 genotypes were isolated from patients with mild symptoms, whereas the G222 variant originated from a fatal case.
Hemagglutinating properties with erythrocytes from different animal species and replication properties in cell lines varying in α2,3SA and α2,6SA receptor expression confirmed a stronger α2,3SA binding of the G222 genotype in comparison to the D222 and E222 variant. However, infection of female BALB/c mice with a dose of 10^6 $TCID_{50}$ of each virus did not revealed significant differences in terms of maximum body weight loss, lung histopathology, and lung weight changes (both at d4 and 21 p.i.) between the isolates. The strongest impairment of general condition was recorded after infection with both the D222 and E222 variant. Compared to other genotypes G222 caused an intermediate lung viral titer on d4 p.i.
In summary, the stronger binding of pH1N1 with G222 to α2,3SA was confirmed. However, this mutation was not found to be associated with severe pathogenicity in mice.

P 12

Growth of porcine influenza viruses in differentiated respiratory epithelial cells

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Keywords: respiratory epithelium, precision-cut lung slices, porcine influenza viruses

We have recently reported precision-cut lung slices (PCLS) as a model system to analyze the infection of porcine influenza viruses in their natural target cells (Punaydarsaniya et al., PlosOne 6:e28429, 2011). Comparison of a porcine virus of the H3N2 subtype with an avian virus of the H9N2 subtype revealed differences in the virulence as indicated by various parameters: (i) duration of the growth cycle, (ii) amount of infectious virus released into the supernatant, and (iii) extent of the ciliostatic effect. Here we compared three porcine viruses of the three subtypes currently prevalent in the swine populations (H3N2, H1N1, H1N2). Comparison of the data with pathogenicity data in pigs and mice reveals which of the above parameters is the best indicator of the pathogenicity of porcine influenza viruses.

P 13

Suppression of the influenza virus induced type I interferon response by co-infection with *Staphylococcus aureus* leads to enhanced virus replication

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Keywords: Influenza A viruses, S. aureus, co-infection

Influenza A viruses (IAV) are the causative agents of severe respiratory diseases, in some cases even leading to death. The majority of these fatal outcomes are linked to secondary bacterial pneumonia, mainly caused by *Staphylococcus aureus* (*S. aureus*). The interplay of viruses with bacteria during infection, even though long known, is hardly characterized on molecular level.
Interestingly, infection with *S. aureus* as well as IAV induces similar cellular signal-transduction patterns, such as activation of MAP kinase cascades, the NF-κB pathway or the phosphatidylinositol-3 kinase. Since nothing is known about the regulation of these pathways during co-infection with both pathogens we assessed how *S. aureus* may interfere with IAV-induced signalling *in vitro*.
Our results show enhanced IAV replication upon co-infection with *S. aureus.* In correlation to this we observed inhibition of IAV-induced type I interferon (IFN) response in the presence of *S. aureus.* These findings are reflected on the level of IRF-3- and IFNβ-promoter activity. Furthermore, mRNA expression of IFNβ and the strictly IFN-dependent genes MxA and OAS is diminished. Therefore, based on our data, an IFN suppressive effect of *S. aureus,* that in turn leads to enhanced IAV replication, could be postulated.
Here, we will provide deeper insights into the interplay of IAV and *S. aureus* regulating antiviral cellular strategies, resulting in enhanced progeny IAV.

P 14

Influenza A virus does not encode a tetherin antagonist with Vpu-like activity and induces IFN-dependent tetherin expression in infected cells

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Keywords: influenza virus, tetherin, release

The interferon-induced host cell factor tetherin inhibits release of human immunodeficiency virus (HIV) from the plasma membrane of infected cells and is counteracted by the HIV-1 protein Vpu. Influenza A virus (FLUAV) also buds from the plasma membrane and is not inhibited by tetherin. Here, we investigated if FLUAV encodes a functional equivalent of Vpu for tetherin antagonism. We found that expression of the FLUAV protein NS1, which antagonizes the interferon (IFN) response, did not block the tetherin-mediated restriction of HIV release, which was rescued by Vpu. Similarly, tetherin-mediated inhibition of HIV release was not rescued by FLUAV infection. In contrast, FLUAV infection induced tetherin expression in target cells in an IFN-dependent manner. These results suggest that FLUAV escapes the antiviral effects of tetherin without encoding a tetherin antagonist with Vpu-like activity.

P 15

Determinants of the Ebola virus glycoprotein for counteraction of the antiviral host cell factor tetherin

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Keywords: Ebola virus glycoprotein, tetherin, filoviruses

The interferon-inducible antiviral factor tetherin (BST2, CD317, HM1.24) inhibits the release of several enveloped viruses, such as the human immunodeficiency virus type 1 (HIV-1) and the Ebola virus (EBOV). HIV-1 counteracts tetherin by its accessory protein Vpu and critical residues for tetherin counteraction have been defined. To assure efficient particle release, EBOV counteracts tetherin via its glycoprotein (GP). However, the determinants of tetherin counteraction within EBOV-GP and the mechanism of tetherin counteraction by EBOV-GP are unclear. Here, we aim to map the domains and amino acids of EBOV-GP which are crucial for tetherin counteraction. We could previously show that the EBOV-GP_2 subunit interacts with human tetherin and that counteraction of tetherin is conserved between the GPs of the 5 EBOV species and the Marburg virus GP. Alignment of the amino acid sequences of the six filoviral GP_2 subunits identified conserved residues. These residues were replaced by alanine in the context of the *Zaire ebolavirus* (ZEBOV) GP. All ZEBOV-GP mutants were efficiently expressed, incorporated into virus-like particles, and mediated entry into target cells. Our initial data suggest that certain residues within the GP_2 transmembrane domain contribute to tetherin antagonism and a comprehensive analysis of all mutants will be presented. Collectively our results indicate that, as for HIV-1 Vpu, the EBOV-GP transmembrane domain might be a determinant for tetherin counteraction.

P 16

Localization of serpin-2 in poxvirus infected chicken chorioallantoic membranes and its impact on red pock phenotype

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Keywords: cowpox virus, serpin, CrmA

The serine protease inhibitor (serpin) CrmA, a virulence and host range factor of cowpox virus (CPXV), is long known to be necessary for the red pocks phonotype on chorioallantoic membranes (CAMs) of embryonated chicken eggs. CrmA was shown to interfere with caspase 1 activation thus inhibiting apoptosis. Other Orthopoxviruses also encode homologue proteins with anti-apoptotic features, but most such as vaccinia virus (VACV), do not induce red pocks on infected CAMs.

We used full length bacterial artificial chromosome clones of CPXV strain Brighton Red and of modified vaccinia virus Ankara (MVA) to generate mutant viruses. In previous work we were able to show that the first start codon is crucial for function of CrmA and that the protein alone is not sufficient to induce the red pock phenotype. With the newly generated set of mutants we could demonstrate that the P1-aspartate in the reactive center loop but not the proposed CPXV-specific s1'A loop is crucial to induce hemorrhagic lesions and to confer endothelial localization of CrmA. Whilst the introduction of CrmA alone into MVA did not recover the red phenotype, the SPI-2 protein of VACV was sufficiently complementing the deletion of its homologue in CPXV. Interestingly, immunohistological staining of VACV infected CAMs showed endothelial localization of SPI-2, despite their white pock phenotype. An anticipated effect of the ankyrin repeat protein BR213 on induction of hemorrhagic lesions could not be proven.

P 17

Hantavirus N protein associates with lipid rafts and F-actin and virus is released apically

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Keywords: hantavirus, hemorrhagic fever, release

Hantaviruses, rodent-borne members of the Bunyaviridae family, infect polar epithelia and endothelia. However, the detailed mechanism of entry, assembly and budding was so far not analyzed in polarized cell-culture models. Therefore, we examined the trafficking and release of hantaviruses in polarized MDCKII cells and human primary renal epithelial cells (HREpC).

We analyzed MDCKII cells for the presence of the hantaviral receptors CD55 and integrin $\alpha V\beta 3$ and their susceptibility for hantavirus infection. MDCKII cells express both receptors and are permissive for Old World hantaviruses. Transwell-cultured polarized MDCKII cells were infected with hantavirus Puumala and Hantaan via the apical surface. Supernatants were collected from the apical and basal chamber and analyzed for the presence of viral particles by detection of N protein by Western blot and by inoculation on Vero cells with subsequent monitoring of reinfection. N protein was only detectable in supernatants collected from the apical chamber and cells were only infected by inoculation with supernatant from the apical side. The analysis of the subcellular localization of hantaviral proteins revealed the association of hantaviral proteins with lipid rafts and F-actin. The disruption of these structures interferes with virus assembly.

Together, we demonstrate that hantaviruses require the actin cytoskeleton and lipid rafts for assembly and particles are released from the apical surface of polarized epithelial cells.

P 18

Matrix-based intraviral yeast-two-hybrid (Y2H) analysis of protein-protein interactions of the human coronavirus NL63 ORFeome

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Keywords: coronavirus interactome, Y2H, HCoV-NL63/SARS-CoV

Human coronaviruses account for significant hospitalizations for children under 18 years of age, the elderly and immunocompromised individuals. HCoV-NL63 is one of five human pathogenic CoV identified to date causing about 5 – 10 % of the acute respiratory diseases (for review see Abdul-Rasool and Fielding, The Open Virology J. 2010, 4, 76-84). While HCoV-NL63 is mainly associated with mild respiratory symptoms, a related human Coronavirus, SARS-CoV caused a worldwide epidemic in the years 2002 to 2003, resulting in “Severe Acute Respiratory Disease” with a mortality rate of about 10%.

In order to gain more insight into functions, modes of action and interdependencies of all the different viral proteins, we recently had cloned the SARS-CoV orfeome as the first prototypic coronaviral approach to whole-genome analysis. (von Brunn et al., 2007, 2, e459). Our current approach to gain insights into the different pathogenicites of the highly aggressive SARS-CoV and the “mild” HCoV-NL63 is to compare the protein-protein interactomes of both viruses. In analogy to the SARS-CoV study we generated Y2H libraries of the HCoV-NL63 proteome including full-length proteins and subfragments with a total number of 45 test constructs and screened them for intraviral protein-protein interactions. To obtain an as complete-as-possible dataset, we used a recently developed Y2H vector system that allows pairwise testing of all combinations of N- and C-terminally tagged bait- and prey fusions in an array based screen (Stellberger et al., 2012, MethMolBiol. 815:277-288). Here we

report on the intraviral HCoV protein interactomes and the comparison of the interactomes of both viruses.

P 19

Structural basis of modulation of host-protein synthesis by a virus: The SARS-unique domain binds Paip1

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Keywords: virus-host interactions, SARS coronavirus, protein synthesis, poly(A)-binding protein-interacting protein 1

The severe acute respiratory syndrome (SARS) coronavirus is more pathogenic for humans than any other coronavirus. The SARS-unique domain (SUD) is only encoded by SARS-coronavirus (SARS-CoV) but absent in other coronaviruses, and therefore possibly associated with the extraordinary virulence of SARS-CoV [1]. To establish a stable infection of the host cell, viruses have to interfere with cellular processes such as production of inflammatory cytokines and antiviral type-I interferons, and they have to modulate apoptosis as well as protein synthesis. Here we used a yeast-two-hybrid system approach and other methods (such as CoIP, ITC etc) to identify possible interaction partners of the SUD among the human proteome, and found PABP-interacting protein 1 (Paip1) as a partner. As the 3-D structure of this protein was unknown, we determined the one of the middle domain of Paip-1 by X-ray crystallography and found the molecule to comprise 5 HEAT repeats [2]. Next, we crystallized the complex between Paip-1M and the N-terminal domain of SUD and determined its structure at 4.1 Å. Interactions such as this are of interest as targets for new types of antivirals.

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P 20

Generation and characterization of an open reading frame 3a deleted SARS-Coronavirus using a reverse genetic approach

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Keywords: SARS-Coronavirus, accessory proteins, ORF3a

SARS-Coronavirus (SARS-CoV) encodes several accessory genes that are interspersed among the viral structural genes and have largely unknown functions. One such gene is the open reading frame 3a (ORF3a), which encodes protein 3a (p3a) and is highly conserved among the coronavirus groups.P3a is a structural viral protein with putative potassium channel function and it was shown to be responsible for Golgi membrane rearrangement in infected cells. Previous studies have reported no visible growth differences between wild type (WT) and a deletion mutant (ΔORF3a) at high infectious doses.

In this study we have deleted ORF3a in an existing infectious cDNA clone of SARS-CoV to investigate the functional aspects of p3a in full context of the replicating virus. By site-directed mutagenesis the start codon of ORF3a was replaced by two adjacent stop codons, followed by reconstruction into the full length clone. Infectious viruses were rescued and viral genomic RNA integrity was confirmed by whole genome sequencing. In contrast to earlier research, we could show attenuation of ΔORF3a in Vero FM cells at low multiplicities of infections (MOI=0.001 to 0.00001). Under these conditions ΔORF3a replicated less efficiently, caused reduced cytopathogenic effects and generated smaller plaques than WT.

Future studies will include 1) the identification of critical amino acid residues that are responsible for observed growth differences and 2) passaging of ORF3a mutant viruses to induce second site mutations for the elucidation of intraviral protein interactions.

P 22

Molecular mechanism of *Pasteurella multocida* toxin to activate heterotrimeric G proteins

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Keywords: Pasteurellosis, bone, protein toxin

Pasteurella multocida is an opportunistic pathogen, which is a frequent resident of the nasal pharyngeal space of animals. An infection of humans normally arises from scratches and bites by domesticated animals such as cats and dogs. Under special conditions infection of pigs with *P. multocida* leads to an atrophic rhinitis, which is characterized by the atrophy of nasal turbinate bones accompanied by a shortening and twisting of the snout. The causative agent of atrophic rhinitis is the bacterial protein toxin PMT (*Pasteurella multocida* toxin) produced by serogroups A and D of *P. multocida*. After entering the cell the 146-kDa toxin activates various signal transduction pathways by activating heterotrimeric G proteins of the $G\alpha_q$, $G\alpha_{13}$ and $G\alpha_i$ family. Here we present the molecular mechanism of PMT to activate heterotrimeric G proteins. PMT deamidates an essential glutamine residue of the α-subunits of the $G\alpha_q$, $G\alpha_{13}$ and $G\alpha_i$ family. Thereby the toxin inhibits the GTPase cycle of heterotrimeric G proteins, in particular the GTPase reaction, which converts the active G protein to its inactive form. Therefore the toxin arrests the G protein in a constitutive active state.
Although the influence of PMT in the pathogenesis of human pasteurellosis is not studied so far, the identification of the molecular mechanism of PMT will facilitate the understanding of the impact of PMT on bone development and especially the pathophysiology of atrophic rhinitis.

P 23

The microRNA-34a targets Selectin P Ligand upon intestinal *Salmonella* infection

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Keywords: microRNA, SELPLG, Salmonella infection

Recent studies identified microRNAs (miRNAs) as key regulators of intestinal development and immunological processes, showing that their dysregulation may lead to intestinal disease.
However, most miRNA-mRNA-interactions are still to be deciphered by functional analyses. The aim of this study was to identify regulated miRNAs and their targets during *Salmonella* infection and probiotic treatment using pigs as a model. Pigs serve as a suitable model for studying intestinal development or disease because of their close phylogenetic distance to humans.
Our results showed dysregulation of Selectin P Ligand (SELPLG) in ascending colon upon *Salmonella* infection in piglets. Interestingly, a restitution of this gene was observed in the probiotic treated group. MicroRNA-target prediction using bioinformatical tools revealed miR-34a as a potential regulator of SELPLG. Interestingly, microarray analyses of same samples pointed out miR-34a to be up-regulated in piglets upon *Salmonella* infection.
The interaction between miR-34a and its target SELPLG was verified by luciferase reporter assays. The porcine intestinal epithelial cell line IPEC-J2 and the human cell line HeLa were each co-transfected with reporter-plasmids harbouring the identified porcine or human SELPLG target site and miR-34a mimic. Reporter-plasmids harbouring the mutated target site as well as non-sense miRNAs served as controls. In both cell lines reporter assays resulted in consistent and significant down-regulation of luciferase activity under the control of SELPLG target site. Our ongoing work concentrates on the cellular regulation of SELPLG protein after miR-34a mimic and anti-miR treatment.

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P 24

Host-pathogen-interactions of different chlamydial strains and primary avian macrophages

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Keywords: Chlamydia, avian macrophages, host-pathogen-interaction

Background: Within the family of *Chlamydiaceae,* there are species that are remarkably different in host adaption and virulence.
Aim: The aim was to study host-pathogen-interaction of three chlamydial strains and primary avian macrophages at the molecular level.
Animals and Method: A cell culture system for primary macrophages from spleen of chickens aged between three and six month has been established. After six days of culture, the differentiated macrophages were infected with *Chlamydia (C.) psittaci, C. pneumoniae* or *C. muridarum*. At different times after infection, the percentage of infected cells was determined by light and fluorescence microscopy. mRNA expression levels for iNOS were monitored by quantitative real-time RT-PCR.
Results: The cultured primary avian macrophages showed a high rate of auto-fluorescence within the cytoplasm. Different percentages of *Chlamydia*-infected macrophages were found depending on the multiplicity of infection (1 and 2), the incubation time (4 h, 24 h or 48 h) and the chlamydial strain used. After *C. pneumoniae* and *C. psittaci* infection, transcription of iNOS mRNA was increased.
Prospect: In further studies, chlamydial DNA copy numbers per macrophage, as well as specific virulence- and immune-related proteins will be examined to characterise host-pathogen-interactions in more detail.

P 25

Ultramorphological features of the *Chlamydia abortus* developmental cycle

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Keywords: Chlamydia abortus, transmissionelectronmicroscopy, developmental cycle

Chlamydia (C.) abortus is the causative agent of abortions in small ruminants leading to considerable economic damage to the agriculture industry worldwide. The zoonotic pathogen can be also transmitted to humans posing a life-threatening health risk in pregnant women.
Transmissionelectronmicroscopy (TEM) studies of this pathogen using high pressure freezing as fixation method revealed new ultramorphological features concerning the chlamydial cell cycle. Strikingly, the formation of small membrane-bordered vesicles in the periplasmatic space of reticulate bodies was observed. Concerning the time point of their formation and the fact that they harbour chlamydial components as shown by immunogold labelling these vesicles might be morphological correlates of an intermediate step during the process of redifferentiation of reticulate bodies into elementary bodies at the end of the developmental cycle. Further, accumulation and even coating of whole inclusion bodies by multiple bilayered lamellae of membranes was observed.
The formation and fusion of multiple chlamydial inclusions within the host cell were analyzed. The putative type 3 secreted virulence factor CAB821, which has been characterized by expression studies at both, mRNA- and protein-level might play a role in the fusion process. Analysis of the subcellular localization of CAB821 at different time points of the growth cycle showed its enrichment at opposing membranes of multiple inclusion bodies.

P 26

In vitro studies on chlamydial virulence and host response of primary macrophages

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Keywords: chlamydia, human macrophages

BACKGROUND: The family of *Chlamydiaceae* comprises nine different species, each displaying different host adaption and virulence potential.
AIM: The aim was to examine the virulence of three different *chlamydial* strains (*Chlamydia (C.) psittaci, C. pneumoniae, C. muridarum*) in connection with the immune response of host cells.
METHOD: First, a cell culture system for primary human monocyte-derived macrophages was established. Buffy Coats from human donors were provided by the University Hospital of Jena, Institute for Transfusion Medicine. Different cell concentrations, culture flasks, 6-wells, FKS', supernatants new seeded, serum coated wells, glass petri dishes, autoplasma, extra coated 6-wells, human sera and M-CSF were tested. Characterisation of cultured macrophages was done by microscopy and flow cytometry.
RESULTS: For the best suitable culture method, 2x10^7 mononuclear cells/ml are seeded onto extra coated 6-wells with RPMI 1640 without indicator (containing 5% FKS, 5% HuS-Si070, Pen/Strep). The supernatant is reused after 90 min. The method resulted in very good confluence and typical macrophage morphology. Flow cytometric analysis showed about 90% macrophages.
PROSPECT: The established culture of human macrophages will be used for infections with different *chlamydial* strains on day 8 after seeding. To assess molecular aspects of *chlamydial* pathogenesis and host response, the cells are harvested for DNA and RNA isolation in specific time intervals.

P 27

Characterization of the interaction of *C. psittaci* with the Golgi apparatus

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Keywords: Chlamydia, Psittacosis

Chlamydiacae are obligate intracellular bacteria causing a wide range of diseases in human and animals. *Chlamydia psittaci*, a pathogen primarily of parrots and poultry has significant zoonotic potential. In humans symptoms of the infection range from fever to severe pneumonia in humans, whereas infections with the human pathogen *C. trachomatis* mostly remain symptom free.
C. trachomatis has been shown to interact with the Golgi apparatus. This interaction is improved by *Chlamydia*-induced fragmentation of the Golgi apparatus into smaller ministacks, leading to increased infectious progeny.
In this project we investigate the relevance of the Golgi apparatus for infections with *C. psittaci.* First data suggest that in *C. psittaci* infections the fragmentation of the Golgi apparatus is significantly delayed compared to *C. trachomatis.* Comparative expression of CPAF, the secreted chlamydial protease shown to be essential and sufficient to induce GA fragmentation in human cells, revealed differences in the level of cytotoxicity between the two different species.
Further characterization of these differences will hopefully improve our perception of chlamydial host cell specificity and its relevance to the emergence and severity of zoonotic diseases.

P 28

New findings concerning the dense granule protein GRA9 in *Neospora caninum*

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Keywords: Neospora caninum, dense granule protein

The parasite *Neospora caninum* belongs to the phylum apicomplexa and is very close related to *Toxoplasma gondii*. In *N. caninum* we identified a putative homologous GRA9 to *T. gondii* GRA9. *Nc*GRA9 was shown to belong to the dense granule proteins and these proteins are described to have roles in host-parasite relationship.
In extracellular parasites *Nc*GRA9 was found in a dotted pattern all over the parasite which implies that this protein is located in the dense granules. After invasion into the host cell *Nc*GRA9 is secreted into the parasitophorous vacuole (PV) where it targets to the vacuolar space and the PV membrane. Further, *Nc*GRA9 was identified as one of the excreted-secreted antigens of *N. caninum*. The secretion of *Nc*GRA9 was also monitored by confocal microscopy and at least 6 hours post infection the protein was present in the PV. In addition, *Nc*GRA9 seems to be phosphorylated during or after secretion into the PV. By cell fractionation it was shown that secreted *Nc*GRA9 exists as a soluble and as a membrane bound protein where it might be associated to the membranous tubules and the PV membrane. Inside the dense granules of extracellular parasites *Nc*GRA9 is soluble and also associated to protein aggregates in the core of the organelles.
In sum, it was shown that *Nc*GRA9 is a dense granule protein of *N. caninum* which has many similarities to its homologous *Tg*GRA9.

P 29

Mycobacterium avium subsp. *paratuberculosis* (MAP) infection inhibits cycloheximide-induced apoptosis in intestinal bovine epithelial cells

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Keywords: MAP, Intestinal Epithelial Cells, Apoptosis

Survival and persistence of intracellular bacteria depends on the ability to manipulate host cells, including inhibition of cell death by apoptosis. After oral ingestion intestinal epithelial cells are the first to interact with MAP the causative agent of ruminant paratuberculosis. The aim of our study was to investigate the effect of MAP on viability of intestinal bovine epithelial cells (cell line FKD-R). Furthermore, the capacity of the bacteria to inhibit apoptosis of these cells was assessed.

FKD-R cells were inoculated with MAP (strain ATCC 19689) at a MOI of 100 and cultivated for 3-30 d. Cell viability was evaluated by methyl-thiazolyl tetrazolium (MTT) assay. Apoptosis was examined by light microscopy, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL) and agarose gel electrophoresis of fragmented genomic DNA.

After 10 days of cultivation uninfected FKD-R cells significantly changed cell morphology and succumbed to cell death until day 12 while infected cells formed a closed monolayer and showed no signs of increased cell death. The TUNEL assay revealed that infection reduced the number of FKD-R cells with fragmented chromatin. The number of apoptotic cells induced by 24 h exposure to Cycloheximide (100 µM) was reduced by MAP infection from 38 % to approximately 24 %.

The results indicate that MAP bacteria deploy inhibition of apoptosis for prolonged survival in intestinal epithelial cells upon infection of ruminants.

P 30

Proteome profiling of *Mycobacterium avium subsp. paratuberculosis* isolates obtained from cows with clinical Johne's disease reveals metabolic adaptation in the natural host

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Keywords: protein expression; metabolism, 2D DIGE

The knowledge about the adaptation of pathogenic mycobacteria to the environment in their natural hosts is limited. *M. avium* ssp. *paratuberculosis* (MAP) causes Johne's disease, a chronic enteritis of ruminants and is discussed as a putative etiological agent of Crohn's disease. In the present study we used LC-MS/MS and 2D DIGE approach to compare the protein profiles of MAP prepared form its natural host with the same strains grown *in vitro*. The central metabolism of MAP in the host seems to be driven by β-oxidation of lipids, most probably host cell cholesterol. β-oxidation is accompanied by an alternative TCA pathway in which a type 2-oxoglutarate ferredoxin oxidoreductase might contribute to the conversion of a ketogluturate to succinyl-CoA. Carbon efflux from the TCA cycle might be compensated by the generation of a ketogluturate from glutamate via enhanced degradation of proline. Within the host, metabolism of MAP seems to be accelerated as indicted by an enhanced activity of the PPP. Enhanced ATP generation through respiration gives evidence for an increased demand for energy. Adaptation to antimicrobial host reactions is indicated by enhanced expression of SodA and KatG as well as other chaperon like proteins. In conclusion, this is the first comprehensive study of the metabolism of a pathogenic mycobacterium in its natural host. Our results overall provided a highly specific picture about the metabolic adaptation of MAP in its natural host.

Poster Session Novel methods and diagnostics

D 01

Efficient enrichment of viral nucleic acids for targeted next generation sequencing

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Keywords: Next Generation Sequencing, Lyssavirus, Rabies

Rabies is a worldwide viral zoonosis caused by lyssaviruses. With several tens of thousands human cases every year, it represents a serious public health threat especially in African and Asian countries. With poor surveillance and underreporting, rabies is considered a neglected zoonotic disease. In the last few years several new lyssavirus species were discovered. For diagnostics, phylogenetic analyses and molecular epidemiology it is important to generate full-length sequences covering all lyssaviruses and for the prototype rabies virus (RABV) all geographical areas. In the framework of the BMBF funded joint project "Lyssaviruses – a potential re-emerging public health threat", diverse aspects of lyssaviruses are investigated in cooperation with national and international partner organizations. The objective is to establish sensitive and reliable methods for lyssavirus whole genome sequencing using Next Generation Sequencing (NGS). A central issue in virus sequencing by NGS is to direct the sequencing efforts away from host nucleic acids to the target nucleic acids, i.e. those of the virus. For this purpose, hybridization techniques are frequently used. One drawback of these methods is the high risk of contamination of the sequencing library with the capture oligo sample. We established novel and efficient methods that avoid possible cross contamination. Data about the new method and the efficient enriching of target nucleic acids will be presented. Our method allows not only for full-genome lyssavirus sequencing but is also applicable for general viral sequencing purposes.

D 02

Construction of an infectious Chikungunya virus cDNA clone and stable insertion of mCherry reporter genes at two different sites

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Keywords: Chikungunya, reporter virus, mCherry protein

Chikungunya virus (CHIKV) caused massive epidemics in the Indian Ocean region since 2005. It belongs to the genus Alphavirus and possesses a positive-stranded RNA genome of nearly 12 kb in size. To produce genetically modified viruses for the study of various aspects of the CHIKV life cycle, a reverse genetic system is needed. We generated a T7 RNA polymerase driven infectious cDNA clone of CHIKV derived from the Mauritius 2006 outbreak. Electroporation of *in vitro* transcribed RNA resulted in the recovery of recombinant virus with growth characteristics comparable to the parental strain. Using the established cDNA clone, the red fluorescent marker gene mCherry was introduced into two different sites within the CHIKV nsP3 gene. Both constructs allowed rescue of fluorescent reporter viruses with growth characteristics similar to the wild type virus tested both in BHK and C6/36 cells. The stability of the mCherry expressing viruses was proven by retaining red fluorescence after passaging the viruses and reinfecting BHK and C6/36 cells. Furthermore, mCherry autofluorescence was still detectable after permeabilization of fixed cells. Immunofluorescence analysis revealed partial colocalization of the chimeric nsP3-mCherry protein and viral replication complexes detected via anti-dsRNA antibody.
Thus, the two generated reporter viruses represent valuable tools for easy follow up of replicating CHIKV useful in several applications of CHIKV research without affecting the virus fitness.

D 03

A novel vector to clone influenza A viruses segments for the bi-directional, simultaneous generation of vRNA and mRNA using a *CcdB* selection marker and alternative cloning sites

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Keywords: Influenza virus, cloning vector, reassortment

Reverse genetic methods of influenza A virus require the rescue of infectious virus from transfected cDNA of the eight genomic RNA-segments of influenza A virus. For this the viral RNA-segments are cloned as cDNA into special vectors that allow the simultaneous generation of a Pol1-driven vRNA-like transcript of the segment, as well as the Pol2-driven generation of the according mRNA leading to the expression of corresponding protein(s). One of the most critical points in cloning the viral segments into of such expression vectors is an effective selection of vectors that contain the cDNA. To this point, we constructed a bi-directional vector encoding the lethal *ccdB* gene as a negative selection marker flanked with two AarI restriction/cloning sites between the human Pol1-promoter and the murine Pol1-terminator based on the pHW2000 vector by Hoffmann and Webster. In *E. coli* the *ccd* locus of the F plasmid codes for two gene products, CcdA and CcdB, which contribute to the plasmid's high stability by post-segregational killing of plasmid-free bacteria. The CcdB protein which has a longer half life than CcdA interferes with the DNA-gyrase, while CcdA acts as a repressor of CcdB. Therefore expression of CcdB without CcdA leads to cell death. CcdB protein is lethal for all *E. coli* except the strain DB3.1 (containing gyrA462 mutation). Through this newly constructed cloning vector, we overcome the drawbacks of other available cloning vectors (pHW2000blue) encoding LacZ as a selection marker that can lead to confusing results and which contains poorly digested BsmB1 restriction sites. We established two complete reverse genetic system of H9N2 (A/chicken/SA/CP7/1998(H9N2)) and S-OIV-H1N1 (A/Giessen/1/09 (H1N1)) to study the impact of NS reassortment of HPAIV (H5-and H7-) and of LPAI (H9N2) with S-OIV on the host range, replication efficiency and virus-induced cellular response.

D 04

Development of monoclonal antibodies against SARS-CoV Nsp1

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Keywords: SARS-CoV non-structural proteins, monoclonal antibodies

We are studying coronavirus pathogenesis by generating genome-wide protein-protein interaction maps of the highly pathogenic SARS-CoV and of the „mildly" pathogenic HCoV-NL63 coronavirus orfeomes utilizing Y2H and mammalian protein interaction techniques. These comparative screenings are performed on an intraviral, matrix-based [all virus proteins against each other] (von Brunn et al., 2007) as well as on a virus-host level [all individual virus ORFs against human cDNA libraries] (Pfefferle et al., 2011). Goal is the identificaction of viral and cellular target molecules necessary - and thus suitable for interference with - viral replication.
Technically, the viral ORFs and subfragments thereof were PCR-amplified using high-throughput-compatible, recombinatorial cloning techniques (Invitrogen GATEWAY™). The method is based on the use of the bacteriophage lambda recombinase for cloning of genes into an entry plasmid vector which allows the subsequent cloning into a variety of destination vectors for expression in various bacterial, yeast and mammalian systems.
A major viral target identified is the Nsp1 protein of SARS-CoV interfering with immunophilins and the immunologically important Calcineurin/NFAT signaling pathway. To get a molecular handle on the Nsp1 protein we developed monoclonal antibodies against bacterially expressed Nsp1 of SARS-CoV containing Maltose-binding protein (MBP) or HIS tags. We describe the characterization of the expressed proteins and of some of the antibodies by immunochemical methods.

D 05

Development and application of monoclonal antibodies against nucleocapsid protein and glycoproteins Gn and Gc of the Rift Valley Fever Virus

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Keywords: Rift Valley Fever Virus; monoclonal antibodies; recombinant proteins

Rift Valley Fever Virus (RVFV) is an arthropod-borne Phlebovirus (*Bunyaviridae* family) affecting humans and a wide range of vertebrate hosts. It causes high neonatal mortality in livestock and haemorrhagic fever in humans. There is a great potential of virus spread from Africa to Europe as there are numerous vector competent mosquito species.

The aim of this study was the generation of monoclonal antibodies (mAb) for diagnostic purposes and for studying the RVFV pathogenesis. Therefore, Balb/c mice were immunised with recombinant RVFV proteins (glycoproteins Gn, Gc; nucleocapsid protein NP) and panels of monoclonal antibodies (mAbs) were obtained.

All RVF mAbs were characterized by indirect ELISA, western blotting and indirect immunofluorescence. In summary, we could identify 45 positive hybriboma cell clones in total harbouring a high and specific reactivity against the corresponding antigen in all performed assays.

In a preliminary study, mAbs were tested in an in house competition ELISA (cELISA) using hyperimmune rabbit polyclonal sera. The ability of several antibodies to discriminate clearly between positive and negative sera shows their potential application for a further development of this cELISA for testing field sera.

Further studies will reveal specific epitopes of these mAbs and analyse potential neutralizing activities. Moreover, mAbs will be evaluated for the use in immunohistochemistry to get detailed information about virus spread in infected animals.

D 06

Development and evaluation of an indirect ELISA for detecting antibodies against Gn protein of Rift Valley Fever Virus in small ruminants

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Keywords: Rift Valley Fever Virus; indirect ELISA; small ruminants

RVFV is an important zoonotic pathogen transmitted by mosquitoes and causes large outbreaks among ruminants and humans in Africa and the Arabian Peninsula. The virus causes high mortality in new-borns and abortion in pregnant animals. RVFV has a potential to spread from Africa to Europe, since there is a variety of endemic mosquito species representing competent transmission vectors. Therefore, increasing efforts to develop safe and standardized diagnostic assays for both human and veterinary surveillance are required.

All currently available ELISA assays using recombinant antigens detect antibodies against RVFV nucleocapsid protein (NP). We developed an in house indirect IgG ELISA for small ruminants based on RVFV glycoprotein Gn to determine the immunogenicity and relevance of this antigen.

The validation of the ELISA was carried out by testing almost 2000 African field samples from sheep and goats. All serum samples were also analyzed by virus neutralization test (VNT), which is regarded as the gold standard method for RVFV serological testing. The Gn based ELISA displays a high sensitivity and specificity, both determined in correlation to the VNT results by ROC analysis. Results also show a high correlation between the presence of neutralising antibodies and anti-RVFV glycoprotein Gn antibodies. In summary, this new ELISA can be used as a reliable diagnostic tool for disease surveillance and monitoring programmes.

D 07

Simultaneous identification of DNA and RNA viruses present in pig faeces using process-controlled deep sequencing

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Keywords: viral Metagenomics, 454 Pyrosequencing, pig faeces

Animal faeces comprise many microorganisms including viruses. Only little is known about their diversity. Here, a protocol was established and optimized for purification of viral particles from faeces.
Methods: The genomes of the purified DNA and RNA viruses were amplified and subjected to deep sequencing. The efficiency of the method was monitored using a process control consisting of three phages with different morphologies and genome types. Known amounts of the phages were added to the sample and their abundance was assessed by quantitative PCR.
Results: All of the added phages were detected. In total, 8% of the reads showed significant identities to published viral sequences. The detected reads mainly originated from phages (76%) and mammalian viruses (23%). The most abundant detected porcine viruses were kobuvirus, rotavirus C, astrovirus, enterovirus B, sapovirus and picobirnavirus. In addition, reads with identities to the chimpanzee stool-associated circular ssDNA virus (ChiSCV) were identified. Whole genome analysis indicates that this virus, tentatively designated as pig stool-associated circular ssDNA virus (PigSCV), represents a novel pig virus.
Conclusions: The established protocol enables the simultaneous detection of DNA and RNA viruses in pig faeces including the identification of so far unknown viruses. It may be applied to studies investigating aetiology, epidemiology and ecology of diseases. The implemented process control ensures comparability of the method.

D 08

Detection of hepatitis E virus in experimentally infected domestic pigs and wild boars by immunohistochemistry

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Keywords: Hepatitis E virus, immunohistochemistry, pigs and wild boars

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in humans, primarily in developing countries. However, an increasing number of autochthonous HEV infections have also been found recently in industrialized countries. Mammalian HEV belongs to the *Hepeviridae* family and clusters into at least four genotypes. Genotypes 1 and 2 are observed exclusively in humans. There is increasing evidence for zoonotic transmissions of HEV genotype 3 and 4 from domestic pig and wild boar to humans. HEV is a non-enveloped virus with a positive sense, single-stranded RNA genome encoding three open reading frames (ORF). The nucleocapsid protein encoded on ORF2 harbours the most important immunogenic epitopes. To date, no appropriate HEV cell culture model for diagnostic applications is available.

Here we describe for the first time the immunohistochemical (IHC) detection of HEV antigens in formalin-fixed tissues of experimentally infected wild boars and domestic swine. Antigens were visualized using a polyclonal antiserum raised against recombinant capsid protein (genotype 3). HEV antigens were located primarily in Kupffer cells in the liver of all wild boars and to a lesser extent in domestic pigs. Furthermore, viral antigens were also detected in spleen and in hepatic, mandibular and mesenteric lymph nodes.

IHC studies provide new insights into the HEV pathogenesis, virus persistence in infected individuals and may reveal suitable cell types for the in-vitro propagation of HEV.

D 09

Identification of specific epitopes for serologic West Nile Virus diagnostics

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West Nile Virus (WNV) is a zoonotic virus which belongs to the family Flaviviridae, similar to Yellow Fever Virus or Japanase Encephalitis Virus. The normal transmission cycle is between birds and mosquites but also mammals such as horses or humans can be infected via mosquito bites. Although the viremia in infected mammals is not high enough to allow further transmission of WNV. An infection can lead to severe neurological disease. Specific serologic diagnosis of WNV infection is complicated by the fact that antibodies to WNV-structural proteins display considerable cross-reactivity to other flaviviruses. Due to this problem we expressed the entire WNV-proteome as 180 overlapping fusion proteins in bacteria. Using this library we are performing a serologic screen by studying antibody binding of sera from humans infected with WNV and other flaviviruses. In addition, sequence variations of novel European WNV isolates from different lineages are included.

D 10

Establishment and employment of primary chriopteran organ cultures and differentiated epithelial cells as a platform for the investigation of the interplay between viral pathogens and bats.

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Keywords: bats, primary cell cultures, virus- host interaction

Bats have been shown to host many viruses related to human pathogenic viruses. Most data rely on the detection of viral nucleic acids or the presence of antibodies. The isolation and cultivation of such viruses is difficult and, in the case of coronaviruses has not been succesful so far. The recent establishment of different immortalized bat cell lines has not solved this problem and raises the question if they lack some properties essential for virus propagation. Given the facts that bats are often endangered and comprise a high biodiversity, and that there is no animal model it is difficult to solve this question. Therefore, there is a need to establish cell cultures that can mimic the *in vivo* situation.

We have a variety of permanent bat cell lines from different organs. Individual cell lines were chosen for incubation under conditions that can induce polarized growth and cell differentiation. Additionally, we prepare and cultivate primary organ cultures from the lung and intestine of *Carollia perspicillata*. Such cell cultures were further characterized by antibody staining, measurement of the transepithelial resistance and their susceptibility to viral infection.

D 11

Plaque assay for Rickettsiae in mammalian cell lines

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Keywords: Rickettsia spp., plaque assay

Rickettsiae comprise obligate intracellular zoonotic bacteria. Over the last 20 years the number of newly detected *Rickettsia* spp. vastly increased and most of them represent potential human pathogens. Cell culture systems are prerequisite for isolation and *in vitro* studies of rickettsiae. Regarding antibiotic susceptibility testing or host-pathogen interaction studies quantification of infectious particles is necessary but hindered by the strictly intracellular growth of *Rickettsia*. Clear-cut and repeatable plaque assays have already been described e.g. for *Rickettsia rickettsii*, *R. conorii*, *R. prowazeki*, and *R. typhi*. We used two mammalian cell lines (Vero E6, L929) and three different overlay media (1.25% methylcellulose, 1% agar, 1% agarose) in order to establish a plaque assay useful for several different rickettsiae, e.g. *R. honei* and *R. helvetica*. After 7-20 d of incubation at 32°C, 5% CO_2 cultures were fixed in formalin and stained by crystal violet (1%). Results obtained so far yielded superior assays with distinct plaque formation in Vero E6 cells compared to L929 cells. Agar and agarose overlay resulted in similar results but plaques appeared smaller when using methylcellulose. Currently, titration and back-titration of different *Rickettsia* cultures are performed in order to prove the stability and reliability of this technique.

D 12

Development of a microarray assay for detecting *Coxiella burnetii* the causative agent of Q fever

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Keywords: oligonucleotide microarray, Coxiella burnetii

Q fever is a notifiable zoonosis caused by the intracellular bacterium *Coxiella burnetii*. The primary reservoirs of this highly infectious agent are sheep, goats, and to a lesser extent cattle. In humans, Q-fever causes a flu-like disease with fever, severe headaches and pneumonia. Chronic Q fever may result in severe, sometimes even fatal cardiac disease. In Germany single cases or small outbreaks are regularly encountered.
For rapid and definite identification of *C. burnetii*, we have designed an easy-to-handle microarray assay based on the cost-effective ArrayTube® platform. This oligonucleotide microarray encompasses three chromosomal and two plasmid located target regions. Every target region is amplified with biotin-labeled primers and the PCR products are pooled and applied to the array. For the validation of the new diagnostic assay, we used *C. burnetii* reference strains, numerous isolates from the German Q fever network (Q-Fieber-Verbund), and a multitude of German strains from the National Reference Laboratory for Q Fever.

D 13

Development of a DNA oligonucleotide array as a first step towards molecular typing and virulence profiling of *Vibrio* spp.

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Keywords: Vibrio spp., DNA microarray, emerging infectious diseases

Bacteria of the genus *Vibrio* are gram-negative curved rods, found naturally in marine and estuarine waters. Global warming which leads to elevated seawater temperatures, as well as an increased trade and consumption of seafood, give rise to a growing incidence of vibrioses. In particular, *V. parahaemolyticus* or *V. vulnificus* are Non-Cholerae *Vibrio* spp., which can be isolated from contaminated food or human specimens. In contrast to *V. cholerae*, Non-Cholerae vibrios may cause infections with varying clinical manifestations such as diarrhea, wound infections or sepsis. In order to gain deeper insight into pathogenicity mechanisms and the function of specific virulence determinants of these emerging pathogens, we have developed a DNA microarray which enables us to establish molecular fingerprints of human, environmental and food isolates of Non-Cholerae *Vibrio* spp. The oligonucleotide array uses biotin-labeled amplicons of target sequences and an enzymatic precipitation to detect probe hybridization. To define and optimize test conditions, a first edition of the microarray was produced and evaluated with completely sequenced *Vibrio* spp. type strains. Currently, the array consists of 68 probes enabling us to detect 24 target genes of different *Vibrio* spp.. Finally, we aim to establish a typing array which allows for quick precise and cost effective evaluation of the virulence potential of Non-Cholerae *Vibrio* spp. as well as strain typing by using molecular fingerprints.

D 14

LipL32 – examination of its usefulness to diagnose leptospirosis in pigs

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Keywords: porcine leptospirosis, LipL32, ELISA

Leptospirosis is a widespread disease in the world with a high zoonotic potential. The sensitivity of the momentary gold standard in diagnostics, the microscopic agglutination test (MAT), is controversially discussed.
Current research focuses on the development of an ELISA for the serological diagnosis of leptospirosis. The recombinant protein LipL32 seems to be a promising antigen: it contributes to a large amount of the outer membrane proteins of pathogenic leptospires.
The goal of this study was to test if LipL32 can be used in a screening ELISA for porcine leptospirosis in comparison to the MAT.
Microtiter plates were coated with LipL32. The plate was afterwards blocked with skim milk. After washing the plate, serum was added at a dilution of 1:100. As conjugate, a goat-anti-pig antibody (conjugated with horseradish peroxidase) was used. TMB was applied as substrate and the reaction stopped with H_2SO_4.
It is becoming apparent that MAT negative porcine serum samples can be differentiated from MAT positive samples with the recombinant protein LipL32. Even though there is a correlation of the qualitative results (positive / negative), there seems to be no correlation of the quantitative results (MAT titers / OD values). However, the goal of the ELISA wasn't to establish a replicate of MAT titers, but to give reliable results whether an animal has been exposed to leptospires or not.

D 15

Specific gamma-interferon response to recombinant antigens in goats experimentally infected with *Mycobacterium avium* ssp. *paratuberculosis* (MAP)

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Keywords: paratuberculosis, goats, gamma-interferon assay

An advanced gamma-interferon (IFN-γ) assay could be instrumental for the early diagnosis of paratuberculosis in ruminants. To increase the specificity of this assay, four recombinant MAP-proteins were evaluated as potential replacements of johnin purified protein derivative (jPPD), conventionally applied to restimulate peripheral blood mononuclear cells (PBMC).

Thuringian goats (n=26) were inoculated ten times beginning at the tenth day post natum, 16 goats served as controls. PBMC were isolated from blood samples taken at intervals of four weeks and stimulated for 24h with jPPD, concanavalin A or MAP-proteins. IFN-γ was quantified by an in-house ELISA.

Beginning in the third week p.i., PBMC of inoculated goats responded to jPPD with a significantly increased production of IFN-γ in comparison to unstimulated control cells while PBMC of control animals did not. Detected concentrations reached maximum values in week 18 p.i. and decreased afterwards. Mean IFN-γ values of PBMC of all inoculated goats stimulated with MAP-proteins did not differ significantly from unstimulated PBMC throughout. However, PBMC of two inoculated goats responded repeatedly to MAP-protein 3651c. Progression of this response resembled the one induced by jPPD but at a lower level. PBMC of other inoculated goats occasionally yielded positive results.

Single use of MAP-protein 3651c may not replace jPPD in the IFN-γ assay but the protein is a promising candidate for combined application with other antigens.

D 16

MAP and Crohn's disease? – Preliminary results of a study for isolation of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) from human intestine tissue

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Keywords: Mycobacterium avium ssp. paratuberculosis, Crohn's disease

Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis – a chronic, incurable inflammation of the intestinal tract in ruminants. Since the mid-twentieth century, there is a controversial discussion whether MAP is involved in Crohn's disease, a chronic inflammatory bowel disease of humans.
To demonstrate a possible causal association between MAP and Crohn's disease, tissue specimens were taken from Crohn's disease, ulcerative colitis and control patients. The samples were examined for the presence of MAP cells by liquid culture using Mycobacterial Growth Indicator Tube. Due to the possible involvement of cell wall deficient forms of MAP in the etiology of Crohn's disease, the modified veal infusion broth according to Markesich *et al*. (1988) and the Reids A agar with fetal calf serum according to Bull (2010) were additionally used for culture. After twelve and 24 weeks incubation, cultures were examined for the presence of MAP DNA using conventional and real-time PCR, and analyzed by light microscopy after staining with Ziehl-Neelsen.
Preliminary results will be presented: Both, conventional and real-time PCR could detect MAP positive specimens. However, some specimens showed different results, depending on detection method and/or used media for culture.

The researches are supported by the German Federal Ministery of Education and Research (support code: 01KI1003E).

D 17

High resolution melting curve analysis of an internalin B gene fragment for rapid typing of *Listeria monocytogenes*

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Keywords: Listeria, typing, HRM

The ability to accurately track *Listeria monocytogenes* outbreak strains is essential for control and prevention of listeriosis. Due to the drawbacks of current typing techniques we developed a rapid and cost effective method for subtyping of *L. monocytogenes*.
One hundred ninety two *L. monocytogenes* isolates were typed by high resolution melting (HRM) curve analysis of a specific locus of the internalin B (*inlB*) gene yielding 15 specific melting curve profiles. These 15 HRM profiles corresponded to 18 distinct *inlB* sequence types. The HRM curve profiles obtained correlated to the phylogenetic groups I.1, I.2, II.1, II.2, and III.
An advantage of typing by HRM curve profiling is the division of the most frequent serotype isolates 1/2a, 1/2b and 4b responsible for more than 96% of human cases of listeriosis in Austria, into 13 distinct subgroups. This explicit advancement compared to classical serotyping renders *inlB* scanning as a first choice for gross typing prior to more sophisticated and time consuming typing methods.
The method was successfully used for screening more than 100 food isolates during a multinational outbreak of listeriosis in 2009 and 2010 in Austria, Germany and the Czech Republic. The fast availability of results by HRM curve analysis made it an indispensable tool for tentative and rapid assignment of human listeriosis cases to the outbreak.
This inexpensive assay represents an improvement compared to classical serotyping or multiplex PCR typing protocols.

D 18

Immunologic relevance of the secretory antigens of *Toxoplasma gondii* as target structures for B- and T-cell responses

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Keywords: T.gondii, cellular response, humoral response

Protection against the parasite *Toxoplasma gondii* in infected individuals is realized by humoral and cellular immune response. We here analyze the capacity of Toxoplasma-Lysat-Antigen (TLA) and of a range of different recombinant Toxoplasma proteins (GRA1, GRA2, GRA7, GRA9, MIC5) as targets for cellular and humoral immunity in infected and noninfected individuals.

Humoral response was evaluated by Western Blot analysis of human sera, revealing the presence of specific antibodies against different recombinant Toxoplasma proteins: Analyzing sera of seropositive donors, antibodies against GRA2 could be found most frequently (100%), followed by GRA7 (82.4%), GRA9 (47.1%), MIC5 (47.1%) and GRA1 (5.9%). Cellular immunity was investigated by determing T- cell proliferation to TLA or different recombinant antigens. Cells from all infected donors proliferated to TLA whereas no response was observed in cultures of the uninfected control group. Response to the recombinant antigens was variable between individuals and none of the analyzed antigens elicited proliferation in all cultures from seropositve donors. Therefore we are now interested in analyzing a larger panel of recombinant antigens regarding their capacity to induce cellular immune response and thus providing protection against *Toxoplasma gondii*.

D 19

Shiga toxin subtyping of hemolytic-uremic syndrome-associated Enterohemorrhagic *Escherichia coli*

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Keywords: Shiga toxin subtyping, EHEC, HUSEC

Enterohemorrhagic *Escherichia coli* (EHEC) can produce different types of Shiga toxins (Stx), its major virulence factor. Whereas previously only the differentiation between Stx1 and Stx2 was unambiguous, further subtyping relevant for disease outcome prediction was hampered by the lack of an internationally uniform Stx subtyping nomenclature.

Here, we evaluated the novel Stx subtyping nomenclature which is based on all published sequences characteristic for each of the three Stx1 and seven Stx2 subtypes on the amino acid level and is standardized among six international reference centres. Using a standardized subtyping assay consisting of different PCRs for Stx1 and Stx2 subtyping, the novel nomenclature was compared with previous subtyping results (based on PCR and restriction analysis) of the haemolytic uremic syndrome (HUS)-associated EHEC (HUSEC) strain collection.

Of the 42 isolates characterized, 30 were positive for a single Stx and 12 for combinations of at least two different Stx subtypes using the previous nomenclature. The novel assay resulted in equal results in 39 isolates; in two isolates (HUSEC015, HUSEC036), one Stx-encoding gene was lost and HUSEC028, previously described as stx_{2d}, was subtyped as stx_{2b} *due to* point mutations within the *Pst*I site.

Overall, the novel PCR assay facilitated Stx subtyping and will in future enable both comparability of subtyping results and risk assessment of EHEC isolates based on a sound and standardized Stx nomenclature.

D 20

Functional array for the detection of botulinum neurotoxins: a new multiplex endopeptidase-assay

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Keywords: Botulinum neurotoxin, antibodies, multiplex cytometric bead array, functional assay

Based on the complex structure of botulinum neurotoxins (BoNT) and their high toxicity diagnostics of botulism are challenging. Suitable specific detection reagents for the different sero- and subtypes are not commercially available, thus hampering a standardized diagnostic scheme. The current project aims at developing a combined functional and immunological multiplex array which allows for the simultaneous detection of the enzymatic activity of different BoNT sero- and subtypes in a rapid and highly sensitive manner.
In our previous studies high-affinity antibodies against native BoNT were generated and implemented into a cytometric bead array with an immunological sensitivity below 100 pg/mL (Pauly et al., 2009). For the detection of the functional endopeptidase activity of different BoNT we have recently generated novel antibodies in mice and rabbits which detect the specific cleavage of BoNT target proteins, allowing for measuring the functional activity of BoNT. These novel reagents in combination with our toxin-specific antibodies will be implemented into a functional and immunological, multiplex-based approach for the simultaneous detection and measurement of functional activity of different BoNT sero- and subtypes. This assay will be validated for the analysis of BoNT out of complex matrices.

Pauly, D., Kirchner, S., Stoermann, B., Schreiber, T., Kaulfuss, S., Schade, R., Zbinden, R., Avondet, M.A., Dorner, M.B., Dorner, B.G. (2009). Analyst *134*, 2028 – 2039.
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Poster Session Antimicrobial use and resistance

A 01

The first crystal structure of a bat coronavirus protein: Main protease of the alphacoronavirus HKU8

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Keywords: main protease, coronavirus, HKU8, antiviral drug discovery

In the past 7 years, it has been shown that bats are the hosts of a large number of coronaviruses (CoVs; order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*), both of the *Alpha-* and *Betacoronavirus* genera. Rhinolophid (horseshoe) bats and others have been found to harbor CoVs more or less closely related to SARS-CoV, the virus that caused the outbreak of Severe Acute Respiratory Syndrome in mainland China, Hong Kong SAR, Taiwan, Vietnam, Singapore, Canada, and several other countries during the spring of 2003. Recently, SARS-CoV-related coronaviruses have also been found in Europe (Drexler et al., *J. Virol.* **84**, 11336 (2010)).

To understand molecular evolution, it is necessary to look beyond amino-acid sequence similarities; as 3D structure is better conserved than the primary structure. Accordingly, we study the structures of selected proteins from bat coronaviruses by X-ray crystallography. One of our major targets is the main protease (also called 3C-like protease, 3CLpro) of various CoVs, because in addition to the contribution to understanding evolution, these enzymes are proven drug targets for interfering with coronavirus infection (Anand et al., *Science* **300**, 1763 (2003)).

In this study, we will present the first crystal structure of a protein from a bat coronavirus, that of the Mpro of HKU8, an alphacoronavirus discovered in bats in Hong Kong (Chu et al., *J. Gen. Virol.* **89**, 1282 (2008)). The enzyme is found to cleave typical substrates of the SARS-CoV Mpro well, but surprisingly, it is not inhibited by our inhibitors of the latter enzyme. The structural basis of this unexpected behavior will be discussed.

A 02

The flavivirus NS2B/NS3 protease as a target for inhibitors

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Keywords: Dengue virus, West Nile Virus, St. Louis Encephalitis Virus

In the past two decades, mosquito-borne flaviviruses have been spreading from the subtropics to regions with a more moderate climate. This includes Dengue virus (DENV, subtypes 1 - 4) and West Nile Virus (WNV). Another member of the family that so far leads to relatively limited outbreaks is St. Louis Encephailits Virus (SLEV).
We target the essential NS2B/NS3 protease of these viruses in our efforts to design and synthesize broad-spectrum inhibitors inactivating a wide range of flaviviruses. We have determined crystal structures of the enzymes of DENV-2, WNV, and SLEV, both in an open conformation and in the closed, inhibitor-bound form. To achieve this for the DENV enzyme, autolysis had to be decreased by the introduction of several mutations.
The NS3 protein contains a serine protease domain, which cleaves its substrates preferentially at the C-terminal side of double-basic sequences such as Lys-Arg or Arg-Arg. One series of our peptidomimetic inhibitors contains a decarboxylated P1 residue comprising a cyclohexyl and an amidine group. These compounds, which display Ki < 200 nM for the WNV NS2B/NS3 protease, could serve as leads for non-peptidic, broad-spectrum inhibitors of flvivirus proteases.

A 03

Novel role of the antimicrobial peptide LL-37 in the induction and stabilization of neutrophil extracellular traps

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Keywords: neutrophil extracellular traps, antimicrobial peptide LL-37, cathelicidin

The antimicrobial peptide LL-37, a member of the cathelicidin family, has been shown to be present in neutrophil extracellular traps (NETs). However, the function of LL-37 within the NETs is still unknown, since LL-37 loses its antimicrobial activity when bound to DNA in the NETs. The aim of this study was to explore the role of LL-37 in NETs using confocal immunofluorescence microscopy. We could demonstrate that NETs treated with LL-37 were distinctly more resistant to bacterial nuclease degradation as compared to non-treated NETs. Furthermore LL-37 was able to induce the formation of NETs by disruption of the nuclear membrane of stimulated human neutrophils. Biochemical assays utilizing a random LL-37-fragment library indicated that the ability of LL-37 to block nuclease activity is based on its cationic character, whereas the NET-induction is mediated by the hydrophobic character of the peptide. In conclusion we revealed two novel roles of LL-37 in the innate immunity: Besides its bactericidal activity against various pathogens, LL-37 can stabilise neutrophil-derived DNA or NETs against bacterial nuclease degradation and, is able to induce the formation of NETs. Future work will aim to study the species-specific biochemical differences of cathelicidins for their contribution to the ability of neutrophils derived from different animal species to release NETs during zoonotic bacterial infections.

A 04

Veterinary Consumption of Antibiotics in Germany – Representative Monitoring

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Keywords: antibiotic use, farm animals, database

In Germany no valid data are available which would be suitable for a species specific estimation of drug consumption especially regarding food producing animals. In 2007/2008 therefore a feasibility project was conducted to identify the technical preconditions and to develop a concept for a regular monitoring system within Germany as a country with a non-central federal state system. This project now will be continued as a representative monitoring.
In spring 2012, data on antibiotic use in veterinary clinics throughout Germany will be evaluated. The antibiotic use in pigs, cattle, laying hens and poultry will be examined. The antibiotic use of a one year period is recorded retrospectively. Information will be gathered using the forms obligatory by German law concerning the treatment of animals and the delivery of animal drugs to the animal owners by the veterinarian. Data is entered online into a database and stored pseudonymously.
Veterinarians who participate in the study select suitable farms. The veterinarian signs an informed consent and privacy statement and transfers the aforementioned data. This can be done electronically or by hard copy.
The analysis will focus on the evaluation of the quantities consumed, the number of drug applications, as well as the estimate of the average number of treatments per animal in a farm.

A 05

Comparative analysis of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from broiler chickens at slaughter and from abattoir workers

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Keywords: Microarray analysis, resistance genes, typing

MRSA of the multilocus sequence type (MLST) ST398 have been shown to colonize and/or cause infections in humans with exposure to livestock.
In this study, 28 avian and 19 human MRSA isolates from four abattoirs were investigated. MLST and *spa* typing followed standard procedures. Resistance phenotypes were determined by broth microdilution according to CLSI recommendations. Resistance genes were detected by a *S. aureus*-specific DNA microarray or PCR assays.
Of the 28 avian isolates 23 were MLST type ST398 and *spa* type t011, while two and three isolates were ST398/t108 and ST9/t1430, respectively. Among the 19 isolates of the abattoir workers, three ST9/t1430 isolates, two ST1453/t4652 isolates, one ST1454/t238 isolate, one ST398/t034 isolate and 12 ST398/t011 isolates were found. All ST398, ST1453 and ST1454 isolates and three ST9 isolates showed resistance to four to nine classes of antimicrobial agents. Solely MRSA ST9/t1430 isolates from slaughterhouse one (n=3) were only resistant to β-lactams and fluoroquinolones. While the resistance pheno-/genotypes of chicken isolates from the same flock were closely related, they differed from resistance pheno-/genotypes of isolates from workers at the respective abattoir.
The homogeneity of MRSA isolates from the same flock suggests exchange of isolates between the respective animals. The apparent heterogeneity of MRSA isolates from abattoir workers might reflect the occupational contact with animals from numerous chicken flocks.

A 06

Analysis of small resistance gene-carrying plasmids in Methicillin-resistant *Staphylococcus aureus* (MRSA) and other staphylococci

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Keywords: resistance gene, plasmid, MRSA

During the last few years several novel resistance genes have been identified on multi-resistance plasmids from MRSA isolates. The aim of this study was to investigate small plasmids harbouring solely the novel genes *vga*(C), *dfrK* or *apmA*.
Staphylococcal isolate collections from Germany and Portugal were screened for the presence of the novel resistance genes by specific PCR assays. Three plasmids were identified, transferred into *S. aureus* RN4220 and completely sequenced.
The first plasmid, designated pCPS49, had a size of 5,292 bp, a GC content of 36.8%, carried the lincosamide/pleuromutilin/streptogramin A resistance gene *vga*(C) and originated from a porcine MRSA CC398 isolate from Portugal. The second plasmid pKKS966 had a size of 4,957 bp, a GC content of 36.1%, carried the trimethoprim resistance gene *dfrK* and was isolated from a German *Staphylococcus hyicus* isolate. The third plasmid pKKS49 had a size of 4,809 bp, a GC content of 38.6%, carried the apramycin resistance gene *apmA* and was from a porcine MRSA CC398 isolate from Portugal. Besides the resistance genes, open reading frames for plasmid replication, recombination and/or mobilization proteins were identified. The corresponding proteins were next related to proteins from isolates of the genera *Selenomonas*, *Lactococcus*, *Lactobacillus* or *Bacillus*.
In addition to recently described large multi-resistance plasmids, also small plasmids can harbour the recently described novel resistance genes.

A 07

Livestock-associated MRSA CC398 as a cause of human bacteremia in North-Rhine Westphalia

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Keywords: MRSA, livestock, CC398

Livestock is frequently colonized with Methicillin-resistant *Staphylococcus aureus* (MRSA) belonging to the clonal lineage (CC) 398. Since MRSA CC398 is often transferred to persons with direct contact to these animals, monitoring the emergence of MRSA CC398 among human bacteremia isolates is important to estimate their impact on severe human infections.

In North-Rhine Westphalia (NRW), microbiological laboratories were asked to send every first MRSA isolate per patient derived from blood cultures to a central typing facility. All isolates were initially characterized using sequence-based typing of the *S. aureus* protein A gene (*spa*) and testing for Panton-Valentine leukocidin (PVL) encoding genes.

In total, four of 410 MRSA isolates (1%) from bacteremia cases in NRW (collection period January 1st - May 10th 2012) were associated with *spa* types indicative for MRSA CC398. These isolates belonged to *spa* types t011 and t034 (both n=2), did not harbor PVL encoding genes and were found in the Ruhrgebiet area (n=1), the Münsterland region (n=2) and East-Westphalia (n=1).

Although many farmers in the rural parts of NRW are colonized with MRSA CC398 from the livestock reservoir, the overall burden of bacteremia cases due to MRSA CC398 is limited on a federal-state level. This might indicate that nosocomial spread of MRSA CC398 is limited; however, this lineage continues to be imported in NRW hospitals and causes invasive infections.

A 08

Extended-spectrum β-lactamase (ESBL) genes in bovine *Escherichia coli* isolates collected in the GE*RM*-Vet monitoring program 2006-2010

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Keywords: extended-spectrum β-lactamase, cattle, resistance

The aim of this study was to detect ESBL genes in *E. coli* isolates from diseased cattle collected in the national monitoring program GE*RM*-Vet.

A total of 702 bovine *E. coli* isolates was included in the study and tested for an ESBL-producing phenotype following CLSI recommendations. Corresponding ESBL genes were detected by PCR and identified by sequencing. The ESBL-producing isolates were tested for their susceptibility to 28 antimicrobial agents by broth microdilution.

In total, 91 ESBL-producing isolates were identified originating mainly from gastrointestinal tract infections (n=85). In the sampling period (2006/2007), 6.0% (11/183 isolates) ESBL-producing *E. coli* isolates were identified. In the years 2008-2010, 11.6% (23/198), 22.8% (39/171) and 12.0% (18/150) of the isolates, respectively, were ESBL-positive. The ESBL genes $bla_{\text{CTX-M-1}}$ (n=73), $bla_{\text{CTX-M-15}}$ (n=8), $bla_{\text{CTX-M-14}}$ (n=7) and $bla_{\text{CTX-M-2}}$ (n=3) were identified. In addition to β-lactam resistance, resistance to tetracycline, trimethoprim, sulfamethoxazole-trimethoprim, nalidixic acid, chloramphenicol, enrofloxacin, spectinomycin, florfenicol and/or gentamicin was frequently seen.

In this study an increase of ESBL-producing isolates was observed until 2009. However, the types of ESBL genes detected did not change considerably. Furthermore, antimicrobial resistance patterns indicate that co-selection of such isolates may occur during treatment with antimicrobial agents commonly used in veterinary medicine.

A 09

Long-term monitoring of ESBL-producing *E. coli* in animal farms and farm environment

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Keywords: ESBL, E. coli, animal farming

The production of extended-spectrum beta-lactamases (ESBL) by *Enterobacteriaceae* is limiting dramatically the therapeutic options in today's medicine.
Therefore the research consortium RESET, funded by the Federal Ministry of Education and Research, is investigating the emergence and dissemination of these bacteria among humans, animals and their environment. As part of RESET the overall goal of our subproject is the long-term investigation of the prevalence of ESBL-producing *E. coli* in broiler and fattening pig farms and their vicinity.
Of the seven pig and seven broiler farms under investigation we visited five of each to date. Sampling took place at three different stages within the fattening period and included faeces of individual animals as well as the stable environments' interior (pooled faeces, dust, air, boot swabs, litter) and exterior (air, boot swabs, slurry).
In about 70% of broiler and 50% of pig faecal samples as well as in a variety of internal environmental samples phenotypically ESBL-positive *E. coli* were detected. These microorganisms were also isolated from the stables' external surroundings, which suggests a faecal and/or airborne emission.
In summary, it seems that ESBL-producing *E. coli* have a high occurrence amongst healthy pigs and poultry.
The samplings in the remaining farms and the further investigations of the isolates such as the detection of various resistance-genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, bla_{CMY-2}) are currently carried out.

A 10

Introduction of *in vivo* techniques to evaluate the effects of antimicrobial treatment of *Chlamydia psittaci* infections in calves

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Keywords: Chlamydia psittaci, animal model, antimicrobial treatment

Background: Knowledge about antimicrobial treatment of respiratory *Chlamydia (C.) psittaci* infections has been described as insufficient in both human and veterinary medicine.
Aim: The aim was to establish endoscopic *in vivo* sampling methods to evaluate the effects of antimicrobial treatment of experimentally induced respiratory *C. psittaci* infections in calves. In particular, we have focused on the following techniques: Bronchoalveolar lavage (BAL), brush biopsy and lung tissue biopsy.
Animals and Method: Bronchoscopy was performed in the anesthetized calf with a flexible video endoscope at days 4 and 9 after inoculation.
Results:
1. BAL: Lavaging the left basal lobe with 5x20ml isotonic saline was identified as the best suitable method. The obtained bronchoalveolar lavage fluid has been found to be suitable for cytology, FACS analysis, PCR and immunohistochemistry (IHC).
2. Brush biopsy: A total of three brush biopsies of trachea and bronchial tubes provided enough material for further sample preparation (cultivation of *C. psittaci,* PCR, IHC)
3. Lung tissue biopsy: Up to five biopsies have been obtained from the cranial lobe and found to be adequate for PCR, IHC and electron microscopy.
Prospect: The described techniques have been successfully established and are currently being used in ongoing experiments, where the effectiveness of Tetracyclin (monotherapy) and Tetracyclin plus Rifampicin (combination therapy) are tested.

A 11

Fluoroquinolone-resistant enterobacteriaceae in and around chicken and swine farms

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Keywords: fluoroquinolone-resistance, E. coli, multi-drug-resistance, emission

E. coli occurs as a commensal in the intestine of humans as well as animals. However, it is also an important opportunistic pathogen with different clinical manifestations. In recent years, the appearance of multi-drug-resistance due to extended-spectrum beta-lactamases (ESBL) and plasmid-mediated AmpC-beta-lactamase-producing strains often in combination with **fluoroquinolone-resistance** have caused a reduction in the efficacy of antibiotics in combating diseases caused by *E. coli*

Therefore, the research consortium RESET, funded by the Federal Ministry of Education and Research, is investigating the emergence and dissemination of these bacteria among humans, animals and the environment, in this part of the project specifically in broiler flocks and swine farms. During a long-time study prevalence of fluoroquinolone-resistant *Enterobacteriaceae* at different times within one fattening period in various samples originating from the animals and their environment inside the barn as well as from the vicinity of the farm including air samples are investigated. Until now, five out of seven broiler as well as swine farms were analysed three times in one fattening period.

Preliminary results show that at each time point many samples originating from the animals and from the barn interior, even air, were phenotypically suspected of being positive for fluoroquinolone resistant *E. coli*. Furthermore, phenotypically fluoroquinolone-resistant *Enterobacteriaceae* were also found in the vicinity of the barns.

A 12

Resistant *Enterobacteriaceae* and use of antibiotics in farm animals – recent results of a cross-sectional study in Germany

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Keywords: antibiotic resistance, Enterobacteriaceae, ESBL

The research network "RESET" consists of 10 scientific network partners and 5 associated partners. The project includes different studies of human and veterinary medicine. The aim is to analyse the spread and the development of resistant Enterobacteriaceae as well as the ways of transmission.
The Institute of Biometry, Epidemiology and Information Processing of the University of Veterinary Medicine Hannover performs a cross-sectional study in fattening pigs, cattle, dairy cows and broilers in four districts in Germany. Faecal samples and dust samples from 200 farms are investigated for *Escherichia coli* and *Salmonella enterica* carrying resistance genes. Risk factors like housing and management conditions, feeding, hygiene as well as the use of antibiotics in the investigated animal groups are evaluated by a questionnaire.
So far, 53 are farms visited. First results show a high incidence of ESBL-suspicious colonies in all animal species. In the presentation, first results will be presented including an overview of the risk factors corresponding to farm characteristics, contact to animals and hygiene aspects.

A 13

Phenotypic and genotypic characterization of environmental and clinical *Vibrio vulnificus* isolates from the Baltic Sea

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Keywords: MLST, virulotyping, cell assays

Vibrio vulnificus is an opportunistic human pathogen causing severe infections in humans. It is commonly found in aquatic ecosystems, in particular, estuarine environments, marine coastal waters and aquaculture settings. Its occurrence is favored by high temperatures and relatively low salinities. Human infections are primarily a consequence of contaminated seafood consumption or open wound infections, which can lead to septicemia with high mortality rates. In the Baltic Sea region *V. vulnificus* has been implicated in wound infections especially during hot summer months.
Molecular typing systems have indicated associations of *V. vulnificus* genotypes and the source of the strains either environmental or clinical, suggesting that different genotypes possess different virulence potentials. We examine *V vulnificus* isolates from the German Baltic Sea region with genotypic methods like Multi Locus Sequence Typing, PCR virulotyping and sequencing of putative virulence genes. Combined with investigations on virulence-associated phenotypic properties such as serum resistance, cytotoxicity and hemolytic activities our aim is to assess the pathogenic potential of Vibrio vulnificus strains from the Baltic Sea region.

Poster Session New and re-emerging zoonoses

N 01

Establishment of primary airway epithelial cells and immortalized cell lines from adult bats and rodents as a tool for the study of zoonotic and wildlife-derived viruses

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Keywords: airway epithelial cells, zoonotic viruses, cell culture models

Due to new and improved detection methods, an increasing number of viral pathogens have been described recently, most of them occurring in wildlife. Currently there are two main difficulties in studying these novel viruses: 1) attempts to isolate them in cell culture have been unsuccessful so far in almost all cases and 2) experimental studies on the natural host species are limited due to the lack of *in vitro* models.
Several new cell lines from various species have been generated recently, however they were mostly acquired from embryonic tissue. Furthermore, until now there has been no focus to selectively culture certain type of cells, such as epithelial cells.
We therefore established a protocol for the selective culture of primary airway epithelial cells from organ samples of adult animals. Primary cells and immortalized cell lines from two rodent species, *Myodes glareolus* and *Sigmodon hispidus* and two chiropteran species *Eidolon helvum* and *Carollia perspicillata* were successfully cultured under standardized conditions and immortalized for the generation of infinite cell lines. Characterization including confirmation of epithelial origin by immunofluorescence staining, species confirmation by sequencing a consensus region of cytochrome c oxidase gene and screening for putative pathogens was performed. Infection studies with several viruses showed that these cells are permissive for infection and therefore a suitable model for the study of zoonotic and wildlife derived viruses.

N 02

Neuronal cell cultures from bats – toward a model system to study neurotropic viruses

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Keywords: bats, cell culture, virus-host-interaction

Lyssaviruses are the prototype neurotropic viruses that inevitably lead to fatal infections of the brain in humans. It is known that bats harbour those neuroinvasive viruses. Unlike any other mammals, bats can survive lyssavirus infections.

To investigate the virus-host-interaction of neurotropic viruses found in bats, we present a first attempt to generate neuronal cell cultures from the African fruit bat *Eidolon helvum* – the reservoir species of a genotype 2 lyssavirus. For that purpose, primary cell cultures from *Eidolon helvum* foetuses were generated and immortalized by lentiviral transduction of the SV40 large T antigen. By subcloning the immortalized cell culture, it was tried to isolate neuronal cells (neurons and glia cells) for the generation of a stable cell line and further characterization. Our results demonstrate that with this procedure the isolation of initially multipotent neuronal stem cells (MNSC) was successful. Neurons and proliferating glia cells were present in the mixed cell culture and in one subclone as was confirmed by immunofluorescence testing for beta-3 tubulin (neurons) and GFAP (glia cells).

Ongoing work focuses on an optimized protocol to enrich MNSCs prior to genetic manipulation. This will provide us with a powerful tool for studies into virus-host-interaction and virus isolation of neurotropic viruses.

N 03

Molecular evolution of Puumala hantavirus in an endemic region in Lower Saxony

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Keywords: Hantavirus, Puumala virus, endemic region, molecular evolution

Puumala virus (PUUV) is the predominant hantavirus species in Germany causing mild to moderate cases of haemorrhagic fever with renal syndrome (HFRS). Lower Saxony is an endemic region for PUUV infections in Germany. Since the introduction of the German Infection Prevention Act in 2001 the number of hantavirus cases in Lower Saxony was recorded annually and the highest incidence of human PUUV infections was found in the district of Osnabrück. In this district, rodent monitoring was performed since 2005 and revealed a continuing and high prevalence of PUUV-specific antibodies in the bank vole (*Myodes glareolus*) populations. During the hantavirus outbreak in 2010, a seroprevalence of 93-100% was demonstrated in the bank vole populations from four trapping sites. RT-PCR investigations targeting the S-, M- and L-segment confirmed the high prevalence in the bank vole populations during this outbreak. Phylogenetic analyses of the partial S-, M- and L-segment sequences revealed a novel PUUV lineage clearly separated from PUUV lineages in other parts of Germany including those from neighboring regions in Lower Saxony and North Rhine Westphalia. The first almost complete genome sequence of this PUUV lineage from Osnabrück confirmed these initial phylogenetic analyses. In conclusion, the investigations demonstrated a continuing PUUV prevalence in a highly endemic region. Future investigations will have to prove whether

there are associations between changes in bank vole populations, the prevalence of PUUV infections in these populations and the frequency of human infections in these regions.

N 04

Investigations on *Leptospira* in small mammals at National Park Bohemian Forest, Germany

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Keywords: small mammals, Leptospira, PCR

Leptospira are zoonotic agents that are often transmitted to humans by contact to rodents or their urine. In this study we investigated kidney tissue of 400 small mammals trapped at the Bohemian Forest National Park in the year 2010. After DNA-extraction, a real-time PCR including an internal control assay (IAC) was performed to detect pathogenic *Leptospira spp*. Samples that tested positive were also run in a Duplex PCR in order to distinguish *L. kirschneri* from *L. interrogansl* other pathogenic *Leptospira* species. The latter were subsequently sequenced for further characterization. 15 out of the 400 animals tested positive for *Leptospira spp*. in the real-time PCR (3,75%). Differentiation by the Duplex PCR resulted in the detection of 4 *L. kirschneri* and 10 *L. interrogansl* other pathogenic *Leptospira* species, 1 sample could not be determined. Sequencing of the partial *gyr*B gene revealed 9 *L. interrogans* and 1 *L. borgpetersenii*. In comparison to simila studies, the prevalence of Leptospira in the here investigated animals is rather low. 13 of the 15 positive animals were trapped between 350m and 500m.a.s.l. Further investigations on Leptospira-prevalence in the animals and parameters like e.g. altitude will be carried out.

N 05

Rickettsia spp. in rodents and arthropods in recreational areas around Leipzig

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Keywords: Rickettsia, Rodents, Arthropods

Rodents are important hosts of various pathogens which can cause diseases in men and lifestock animals. They may act as reservoir for several pathogens like *Leptospira spp.*, *Rickettsia spp.*, *Babesia spp.* and *Anaplasma spp*. Rickettsia belong to the pathogens which are transmitted by blood-sucking arthropods such as fleas, ticks and mites. There is not much known about the geographical occurrence, spatial and temporal abundance and species composition of *Rickettsia spp.* in rodents and arthropods in Germany. Thus, a total of 78 rodents were captured from August 2010 to June 2011 in the area of Leipzig. Ticks and fleas were collected from them and a screening for the presence of rickettsial DNA was performed using a PanRickettsia real-time PCR. 32% of the rodents (n = 25/78), 42% of the ticks (n = 154/364) and 4% of the fleas (n = 5/126) tested positive for *Rickettsia spp*. Further investigations of the positive rodent samples were conducted by sequence analysis. The high prevalence of Rickettsia in particular in rodents shows that these potential reservoir hosts may play an important role in the maintaining and distribution of these pathogens in nature. Further studies - especially in the rodents - should be performed in order to gain a deeper understanding of the transmission cycles of particular *Rickettsi spp*.

N 06

Mycobacterium caprae in Bavarian Alpine red deer and differentiation from *M. bovis*

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Keywords: Mycobacterium caprae, red deer, differentiation

For livestock and wildlife large animal hosts *M. bovis* and *M. caprae* are the most prominent pathogens within the mycobacterium tuberculosis complex (MTC).
In the Bavarian Alpine region of "Allgäu", an area with plenty of cattle summer pastures also habituated by red deer low but recurrent incidence of tuberculosis is registered. The infections in cattle and red deer were constantly caused by *M. caprae*. However, since in cattle and red deer both *M. bovis* and *M. caprae* infection can occur it is highly desirable to differentiate between the two Mycobacterium species using a reliable single shot PCR.
The presence of *M. caprae* specific single-nucleotide polymorphism (SNP) in lepA- and gyrB-gene was assessed for robust identification of *M. caprae*. To evaluate molecular characteristics of this species, we analyzed 13 *M. caprae* isolates by differential conventional PCR strategies and sequence analysis of PCR-fragments. The isolates originated from Bavaria, Germany and represent a five year monitoring period. Indicative SNP were detected in all isolates.
To gain an overview about infected red deer the hunting areas in the region of Allgäu and Karwendel mountains were selected for post mortal monitoring in hunting seasons 2009/10, 2010/11 and 2011/12. To date 422 collected specimens were analyzed and five samples were detected as *M. caprae* positive. Interestingly, one infection with *M. caprae* in the absence of macroscopic lesions (non visible lesion, NVL) was diagnosed. Current results of the monitoring will be reported.

N 07

Identification of amino acids in the SARS-coronavirus spike protein which are important for activation by type II transmembrane serine proteases

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Keywords: SARS-coronavirus, type II transmembrane serine proteases

The viral spike protein facilitates host cell entry of the severe acute respiratory syndrome coronavirus (SARS-CoV). Activation of SARS-S by host cell proteases is essential for viral infectivity and it has been shown that the endosomal protease cathepsin L can activate SARS-S by cleavage. Recent studies indicate that the type II transmembrane serine proteases (TTSPs) TMPRSS2, TMPRSS4 and HAT can also activate SARS-S, at least for cell-cell fusion, and that TMPRSS2 activity allows for cathepsin L-independent virus-cell fusion. However, the TMPRSS2 cleavage sites in SARS-S are unknown. We mutated potentially protease sensitive sites in SARS-S, R667, T678A, 758-761, K672L and R797, and functionally analyzed the S-protein mutants in order to identify residues important for activation by TMPRSS2. All mutants were efficiently expressed and mediated robust cell-cell fusion in an experimental setting independent of TTSP activity. Within initial experiments, mutation of R797 decreased activation of SARS-S by TMPRSS2 and TMPRSS4 but had little effect on SARS-S activation by HAT, indicating that the mode of cleavage activation of SARS-S might differ between TTSPs. In contrast, mutations of T678A, 758-761, K672L and R667 did not appreciably impact SARS-S activation. It is currently under investigation if the mutations analyzed impede SARS-S cleavage and modulate SARS-S activation by TMPRSS2 for virus-cell fusion. The results of these studies will be presented.

N 08

Pyrosequencing of Bhanja virus and Palma virus implicates the existence of a new distinct clade of Bunyaviruses

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Keywords: Bunyaviridae, Bhanja virus, Palma virus

Bhanja virus (BHAV) and Palma virus (PALV) are tick-borne Arboviruses of the Bhanja antigenic group, one of seven groups of Bunyaviruses so far unassigned to any genus. These viruses have been isolated from India, various parts of Africa, former USSR and Europe. Confirmed vertebrate hosts are sheep, goat, cattle, African hedgehog *Atelerix albiventris*, and African ground squirrel *Xerus erythropus*. The viruses do not cause apparent infection in adult animals but are pathogenic for young ruminants causing fever and encephalitic symptoms. Several cases of Bhanja group virus febrile illness have been documented in humans, with symptoms including photophobia, vomiting, meningo-encephalitis, and pareses. In addition, experimental encephalitis was produced in rhesus monkeys.
In order to elucidate the phylogenetic relationship of these viruses within the Bunyaviridae family we determined the genome of BHAV and PALV via pyrosequencing. We determined approximately 97%, 99% and 72% of the S-, M-, L- segments of BHAV and 100%, 99% and 99% of the S-, M-, L- segments of PALV, including the complete coding regions for the Nucleocapsid proteins, the Glycoprotein precursors as well as the coding regions for the RdRP's. The 3' terminal sequences of the L–segment of BHAV were missed.
Supported by high bootstrap values phylogenetic analysis groups these viruses together with the new tick-borne Phleboviruses described in China last year provisionally termed SFSTV according to the severe fever and thrombocytopenia syndrome they elicit. While the L- and M- segment sequences of BHAV and PALV and SFSTV form distinct subclades among the Phleboviruses, the S-segment sequences are unique and more akin to Nairovirus sequences. This unexpected mix of Phlebovirus and Nairovirus like segments confirms the past difficulties in assigning these viruses to a particular genus by serological methods.

N 09

STLV antibody prevalence in urine and feces of free living great apes

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Keywords: STLV, apes, non-invasive samples

Simian T-lymphotropic virus type 1 (STLV-1) has recurrently been transmitted from nonhuman primates to humans, presumably through hunting and consumption of primate bushmeat including apes. Therefore, mapping of STLV-1 in wild primate populations is important to assess risk areas and species. Here, we compare rates of STLV-1 antibody prevalence in feces and urine from wild living great apes. We establish a total of 438 Western Blot profiles from bonobos (*Pan paniscus*), orangutans (*Pongo pygmaeus* sp.), western lowland gorillas (*Gorilla gorilla gorilla*) and multiple populations of chimpanzees (*Pan troglodytes* spp.). While no antibodies against STLV-1 were detected in gorillas, orangutans and bonobos, western chimpanzees exhibited an average prevalence of 48% and central and eastern chimpanzees of 11% and 16%, respectively. These data hint at chimpanzees being an important reservoir for zoonotic STLV-1, particularly in western Africa.

N 10

First isolation of an *Entomobirnavirus* from insects

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Keywords: Entomobirnavirus, mosquitoes, RNAi

Drosophila X virus (DXV), the prototype Entomobirnavirus, is a paramount RNA virus model. Its origin is unknown, and so is that of the only other entomobirnavirus, Espirito Santo virus (ESV). We isolated an entomobirnavirus tentatively named Culex Y virus (CYV) from hibernating *Culex pipiens* complex mosquitoes in Germany. CYV was detected in three pools consisting of eleven mosquitoes each. Full genome sequencing and phylogenetic analyses suggested that CYV and ESV define one sister species to DXV within the genus Entomobirnavirus. In contrast to the laboratory-derived ESV, the ORF5 initiation codon AUG was mutated to $_{1927}$GUG in all three wildtype CYV isolates. Also in contrast to ESV, replication of CYV was not dependent on other viruses in insect cell culture. CYV could provide a wildtype counterpart in fields so far relying on DXV.

N 11

Investigation of cross-species transmission by DNA Viruses in a predator-prey system

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Keywords: DNA Virus, Zoonosis, Species specificity

Transmission of viruses from nonhuman Primates (NHP) to humans through hunting, handling and consumption of primate meat is a major concern. Infections with various DNA viruses have been described for a vast majority of NHP, and co-infections frequently occur. It is generally assumed that DNA viruses are species specific, however, there are known exceptions. Here, we investigated a natural predator-prey system, i.e., chimpanzees (predator) and colobus monkeys (prey) for evidence of transmission in Côte d'Ivoire, Africa. By performing degenerate PCR targeting conserved viral genes, several distinct and previously unidentified, as well as known herpes-, adeno- and polyomaviruses were detected in organ and urine samples of the Western chimpanzee *(Pan troglodytes),* Western Red Colobus *(Piliocolobus badius)* and Black and White Colobus *(Colobus polykomos)*. Specific primers targeting different colobus virus sequences were then used to check for the presence of colobus viruses in chimpanzee samples. Phylogenetic analysis based on the novel viruses found in this study, alongside previously characterised NHP and other mammalian viruses, helped to put the issue of cross-species transmission into perspective. There was no evidence of cross-species transmission in the chimpanzees tested so far. This finding might indicate a lack of transmission of the DNA viruses in question through hunting and ingestion.

N 12

Serology in African bats

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Keywords: bat, africa, filovirus

Bats are the presumptive natural reservoir host of Filoviruses as they can be asymptomatically infected by both, Marburg- and Ebolavirus. Monitoring the prevalence of virus and antibodies in bats could therefore provide an efficient, relatively inexpensive method for surveying and predicting outbreak risks. However, antibody detection rates are differing between studies and broad surveys are needed to improve our knowledge on Filovirus occurrence in various regions. We used ELISA to screen blood samples from different bat species collected between 2006 and 2010 in western and central Africa for the presence of antibodies against Marburg- and Ebolaviruses and compared the results to published data. Samples showing reactivity were tested with Western Blot for confirmation of results. Since proper storage (i.e. frozen or cooled) of samples in the field is limited we also tested the impact of different storage conditions for blood samples on the outcome of serological tests.

N 13

Diversity of Paramyxoviruses in neotropical bats

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Keywords: Paramyxovirus, bats, neotropics

Paramyxoviruses comprise leading human viruses, including mumps and henipaviruses. Recently, bats were shown to host major mammalian paramyxoviruses. Paraymxoviruses related to human viruses originated mostly from Old World fruit bats (Megachiroptera), the suborder of fruit eating megabats.

In the Neotropics, megabats do not occur and this ecological niche is occupied by echolocating small bats (Microchiroptera). The diversity of bats in the Neotropics is very high, constituting 24 % of the total bat species worldwide. Therefore, studies in the New World tropics are of great importance for the analysis of diversity, distribution and ecology of bat paramyxoviruses.

In this study, neotropical bats from three different countries were tested for paramyxovirus RNA. Ten different paramyxovirus clades could be detected from various bat species, including sanguirvorous, nectarivorous, fructivorous and insectivorous species from Costa Rica, Panama and Brazil. Bat feeding behaviour was unrelated to distinct virus clades. In contrast to the Old World, Henipavirus-related pathogens were only detected in insectivorous bats.

A high diversity and prevalence of morbilli-related viruses was observed in costarican insectivorous bats. Closely related viruses were detected in the same species through three consecutive years and at different locations, highlighting the importance of microbats for this Paramyxoviridae lineage.

N 14

Highly diversified alpha- and betacoronaviruses in neotropical bats

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Keywords: Coronaviridae, bats, Neotropics

Bats host a broad diversity of coronaviruses (CoV), including close relatives of human pathogens. Despite their species richness and abundance, only very limited data about coronaviruses are available for neotropical bats. We analyzed fecal, blood and intestine specimens from 1,437 bats sampled in Costa Rica, Panama, Ecuador and Brazil for coronaviruses by broad-range PCR. In nine different frugivorous and insectivorous bat species, 42 CoVs were detected. Neotropical bat CoVs were unrelated to established human or animal pathogens, indicating an absence of recent zoonotic spill-over events. Based on RNA-dependent RNA Polymerase (RdRp)-based grouping units (RGU), the 42 viruses represented five different Alphacoronavirus RGUs and one Betacoronavirus RGU. For one Alphacoronavirus RGU from Carollia perspicillata and C. brevicauda, strict association of viral RGU and host genus was shown through a geographic distance exceeding 5,600 km. Our study expanded the maximum bat coronavirus sequence diversity from the Americas to the level previously known for other continents.

N 15

Orthoreoviruses isolated from European bats

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Keywords: vespertilionid bats, mammalian orthoreovirus, virus isolation

We report the isolation of three novel mammalian orthoreoviruses (MRVs) from European bats, comprising bat-borne orthoreovirus outside of South East Asia and Australia and moreover detected in insectivorous bats (Microchiroptera). MRVs are well known to be capable of infecting a broad range of mammals including man. Although they are associated with rather mild and clinically unapparent infections in their hosts, there is growing evidence of their ability to also induce more severe illness in dogs and man. In this study eight out of 120 vespertilionid bats proved to be infected with one out of three novel MRV isolates, with a distinct organ tropism for the intestine. Subsequently, one isolate was analyzed by 454 genome sequencing. The closest phylogenetic relationship of T3/Bat/Germany/342/08 obtained was to MRV strain T3/D04, isolated from a dog suffering on hemorrhagic diarrhea and a simultaneous concurrent canine parvovirus type 2 infection. Interestingly, two of the infected bats also showed hemorrhagic enteritis.
After we recently published a study focusing on the possible evolutionary history of a novel bat adenovirus, we are here reporting the repeated isolation of a virus with the closest phylogenetic relative might be capable of causing or enhancing a severe disease in dogs. These novel reoviruses provide a rare chance of gaining insight into possible transmission events and of tracing evolutionary origins of bat viruses.

N 16

A novel Rhabdovirus from the straw-coloured fruit bat *(Eidolon helvum)* in Ghana

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Keywords: Rhabdovirus, Eidolon helvum, Virusdiscovery

In the recent years an increasing number of novel viruses were discovered in bats. The straw-coloured fruit bat *Eidolon helvum* (*E.helvum*) harbours a high diversity of viruses and combines all characteristics of a small mammalian virus reservoir. They live in large colonies of up to 1 Mill. individuals, have close social interactions and are highly migratory. *E.helvum* is abundant in Sub-Saharan Africa and colonies are often in close contact to the human population. In addition, they are the most hunted bush meat on the African continent. Many rhabdoviruses are known to be harboured by bats however only few were found to infect humans. Those known to infect humans are associated with severe diseases. In this study we isolated a novel rhabdovirus from the spleen of *E.helvum* from Ghana. The full genome of the rhabdovirus was analysed from cell culture supernatant by 454 sequencing. In order to test the prevalence of the virus, *E.helvum* spleens were screened by specific real-time PCR for the novel rhabdovirus. From 494 bat spleens 23 (4.6%) were positive. The presence of antibodies was tested by ELISA and immunofluoresecence assay. Of 352 bat sera 44 (12,5%) reacted positive in both assays. Neutralising antibodies were confirmed in a plaque reduction assay. The potential for a zoonotic spillover of this novel rhabdovirus is yet to be investigated.

N 17

Do human Adenoviruses of species B originate from Gorillas?

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Keywords: human adenovirus B, gorilla, evolution

Human adenoviruses of species B (HAdV-B) are important pathogens causing respiratory tract-, eye- and urinary tract infections. Closely related adenoviruses have been identified previously in gorillas and chimpanzees living in the wild and in captivity. In phylogenetic analysis, these human and great ape HAdV-B form a highly mixed cluster which indicates that inter-species transmission events may have occurred in the evolution of HAdV-B viruses. Great ape adenoviruses are shed frequently in the feces, and in a previous study we noted that gorillas predominantly shed HAdV-B.

Here we analysed in detail feces samples of lowland and mountain gorillas living in their natural habitats in four different regions of Africa for infection with HAdV-B, and wild chimpanzees and bonobos were analyzed for comparison. Using PCR with degenerate and AdV-specific primers a block of 5 genes (pVII to hexon) was amplified. In gorillas HAdV-B were detected with high prevalence, but in chimpanzees they were found only sporadically and none was found in bonobos. In contrast to the human and chimpanzee HAdV-B, the gorilla HAdV-B revealed a high genetic diversity. It is hypothesized that HAdV-B has been transmitted from gorilla to chimpanzees and humans. Bushmeat hunting, handling and consumption as well as

close contact in captive settings may result in further transmissions of HAdV-B from great apes to humans.

N 18

Parapoxvirus (PPV) infection of man: a neglected zoonosis

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Keywords: Parapoxvirus, human infection, molecular differentiation

Pseudocowpox (PCP) or milker's nodule (MN) are common terms for human PPV infections. Nowadays transmission of PPV ovis or Orf virus (ORFV) from small ruminants, mostly sheep, causes the majority of human disease cases. The genus PPV comprises virus species of high stability that infect broken skin or mucous membranes inducing localized lesions (contagious ecthyma , CE) in ruminants. All known virus species are infectious for man with manifestation mostly at the hands (milker's node) and sometimes at other parts of the body where broken skin allows virus entry and replication. Molecular differentiation of PPV species is possible by RFLP and hybridization with species genome specific probes, however reliable PCR protocols are not available for that purpose. Our collection of human and ruminant PPV isolates was used to look after genomic regions suitable for PCR based differentiation. Human cases of PPV infection in Germany are presented in context with clinical outcome, virus isolation and molecular diagnosis linked to the source of transmission.

N 19

Efficacy of two WNV vaccines in large falcons verified by live virus challenge

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Keywords: West Nile virus, vaccine challenge trial, raptors

WNV infections have been observed in wild birds (including raptors) in many Mediterranean countries and even in European countries further north. We showed lately that such infections are fatal or have devastating effects on large falcons. Therefore the vaccination of valuable breeding stocks of raptors and animals in wildlife centres with vaccines that are approved for equines has been proposed. However, data on the antibody stimulation upon vaccination of these birds and protection against a deadly virus challenge are not available to date. We have therefore evaluated two WNV vaccines approved in the EU for use in horses in large falcons and verified the efficacy by live virus challenge. The killed vaccine Duvaxyn® WNV, Fort Dodge (in the EU now Eqip® WNV, Pfizer) and the recombinant live RECOMBITEK®- Equine rWNV vaccine, MERIAL (now approved in the EU as Proteq West Nile) were administered in each two different vaccination schedules. Antibody development and safety during vaccination was recorded. Challenge was performed with subcutaneous inoculation of the WNV lineage 1 isolate NY`99. During the 21 days lasting experiment, serological assays, as well as virological and molecular assays were conducted on oral and cloacal swabs and blood samples. Clinical symptoms were assessed daily. After death or euthanasia animals were necropsied and organ samples analysed by virological, molecular, histological and immunhistochemical methods. The results obtained illustrate that it is possible to stimulate a protective immunity if the appropriate vaccine and vaccination protocol is used.

N 20

Recombinant vaccinia virus MVA as West-Nile virus vaccine for VECTORIE

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Keywords: modified vaccinia Ankara; vector vaccine

VECTORIE (Vector-borne Risks for Europe) is a project funded by the European Commission under the Seventh Framework Programme (SF7) to increase Europe's preparedness for vector-borne emerging diseases like West Nile and Chikungunya Fever.
West Nile Virus (WNV) is a mosquito-borne flavivirus. Intrinsically it is maintained in an enzootic cycle between avian hosts and mosquitos, but it can also infect and cause disease in humans and horses. The virus is widely distributed in Africa, Europe, the Middle East, Asia and since 1999 has spread through North and South America. WNV infects the central nervous system and can cause neuroinvasive disease with the potential for severe courses especially in the elderly and immunocompromised humans. Modified vaccinai virus Ankara (MVA) can be exploited as safe viral vector in medical and veterinary vaccine development.
The aim of our work in this ongoing project is the generation and characterization of recombinant MVA based vaccines delivering WNV antigens. Candidate vaccines are tested for immunogenicity and efficacy.

N 21

Sylvatic origin of St. Louis encephalitis virus

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Keywords: St. Louis encephalitis virus, sylvatic cycles, phylogeography

St. Louis encephalitis virus (SLEV) is the major representative of the Japanese encephalitis serocomplex of flaviviruses in the Americas. However, its geographic and ecological origins remain obscure.
We investigated 3.491 mosquitoes in and around the Palenque National Park, Mexico, and identified the first representatives of sylvatic SLEV in 3 *Culex nigripalpus* mosquitoes. Full genome sequencing revealed that SLEV-Palenque differed from all other SLEV strains by 4.3 – 5.8% on amino acid level. Phylogenetic analyses placed the strains in basal position to all presently known SLEV strains. Virus growth kinetics indicated a lower replication rate and absence of CPE in Vero cells of Palenque-A770 compared to the epidemic MSI-7 strain. Times of existence and geographic locations of phylogenetic ancestors were continuously remodelled in a Bayesian relaxed random walk approach, indicating that the most recent common ancestors of extant SLEV existed in an area comprising southern Mexico and Panama. Expansion of the cosmopolitan lineage occurred in two waves, the first of which marking the evasion of viruses from sylvatic viruses nearby the area of origin, followed by almost parallel appearance of a Southern clade in the area of the Amazonas delta and a Northern clade in the lower Mississippi area. The timing of these major diversification events suggested an involvement of human-derived displacement of viruses.

Poster Session Free Topics

F 01

Generation of a complete cowpox virus single gene knockout BAC library

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Keywords: cowpox virus, BAC, Red mutagenesis

Orthopoxviruses (OPVs) pose an emerging threat for humans. *Variola* virus, the most highly human-pathogenic OPV, was declared eradicated 30 years ago. However, other species of the genus circulate within animal populations, causing zoonotic infections. Cowpox viruses (CPXVs) infect a wide range of vertebrates and are considered to be closely related to the common OPV ancestor, bearing the genetic information to evolve to new species with pathogenic potential comparable to that of more virulent OPVs. Thus, we seek to identify and characterize genes and cellular mechanisms involved in the determination of the host range of CPXV. Starting from a full length BAC clone of the CPXV strain Brighton Red (pBRF), we generated a knockout library containing single gene knockout mutants for each ORF of the virus. All mutant BAC clones were confirmed by RFLP analysis and sequencing. To visualize the early and late phase of viral gene expression independently upon virus reconstitution, we introduced the mRFP gene driven by an early synthetic vaccinia virus promoter into pBRF, alongside the already existing late-expressed eGFP.

Our library enables systematic investigation of the contribution of single CPXV genes to viral infection, replication, pathogenesis and host range determination, and thus provides a versatile resource for the poxvirus community.

F 02

Examining S-Acylation and other effects of mutations in the cytoplasmic tail of influenza virus

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Keywords: Hemagglutinin (HA), S-Acylation, reverse genetics

Influenza virus A's spike protein HA is post-translationally modified by S-acylation at typically three cysteines, one in the transmembrane region (TMR) and two in the cytoplasmic tail (CT). This modification is essential for virus viability. Mass spectrometry revealed that the cysteines in the CT are palmitoylated, while cysteines in the TMR carry stearate.

We hypothesized that the residues surrounding the acylation sites contain consensus signals for S-acylation or determine the fatty acid specificity. To investigate this, we mutated conserved residues in HA's CT to generate recombinant viruses by reverse genetics in the WSN background. Mutation of the conserved glycine-557 (situated three residues downstream of the TMR cysteine) to alanine affected virus growth marginally and mass spectrometry revealed no change in acylation, neither its stoichiometry nor fatty acid selectivity was altered. Mutation of glutamine-560, directly preceding the second acylated cysteine, to glutamic acid resulted in a severely growth-compromised virus. Electron microscopy did not show aberrant morphology of either virus. Mutants G557Stop and I563Q could not be rescued.

The results show that even single-residue changes in the CT strongly hamper virus growth. Further site-directed mutations in this region will clarify whether the amino acid sequence in the vicinity of the modified cysteines or the location of the cysteine relative to the TMR is responsible for differential attachment of fatty acids.

F 03

Generation of virus-like particles based on SARS-Coronavirus structural proteins

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Keywords: CoVLP, Species barrier, Viral entry

The outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002/2003 led to 8000 infections and 800 deaths. Bats are the likely reservoir of SARS-CoV and many closely related CoVs were identified in bats worldwide. This raises the question whether these viruses are able to cross the species barrier and pose a risk to human health. As the isolation of bat CoV has been unsuccessful so far, surrogate systems are required for in-vitro studies of the viral entry process.
In the presented study Coronavirus-like particles (CoVLPs) were generated. A human embryonic kidney (HEK) 293 cell line was established that stably expressed the SARS-CoV membrane- (M) and envelope- (E) protein under the control of a tetracyclin inducible promoter. This cell line was transfected with an expression vector containing SARS-CoV Frankfurt-1 spike (S) protein to produce CoVLPs. CoVLPs were harvested from the supernatant and purified by ultracentrifugation. Successful formation of CoVLPs was proven by Western blot analysis using M, E and S protein-specific antisera.
In future studies CoVLPs will be pseudotyped with S proteins of bat CoVs and equipped with an existing SARS-CoV-replicon containing a *Metridia* luciferase as a reporter gene. This cell culture-based system can be used to assess the potential of emerging bat CoVs to enter cells of non-reservoir species and thus determine the risk of zoonotic transmission.

F 04

Acquisition of new protein domains by SARS-coronavirus: Analysis of overlapping reading frames coding for proteins N and 9b

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Keywords: overlapping reading frames, accessory protein, SARS-CoV, nucleocapsid protein, Orf9b

Acquisition of new proteins by viruses usually occurs through horizontal gene transfer or through gene duplication, but another, less common mechanism is the usage of completely or partly overlapping reading frames. SARS coronavirus (SARS-CoV) presumably used both mechanisms: An example for likely gene duplication are the three consecutive macrodomains X, SUD-N, and SUD-M within non-structural protein 3 (Nsp3). A case of acquisition of a completely new protein through introduction of a start codon in an alternative reading frame is the protein encoded by Orf9b. This gene completely overlaps with the nucleocapsid (N) gene (Orf9a). Interestingly, Orf9b was only acquired after the transmission of SARS-CoV from its bat reservoir to the new human host. We analyzed the evolution of Orf9b in concert with Orf9a using sequence data collected during the course of the SARS epidemic and found that Orf9b, which encodes the overprinting protein, evolved largely independently of the overprinted Orf9a. We also analyzed the available crystal structure of protein 9b (Meier et al., Structure **14**, 1157 (2006)) in terms of flexibility and compatibility with mutations.

F 05

Francisella-like endosymbionts in *Dermacentor* ticks from Southern Germany

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Keywords: ticks, Francisella tularensis, Francisella-like endosymbionts

In Europe, *Dermacentor (D.) marginatus*, *D. reticulatus* and *Ixodes (I.) ricinus* ticks have been reported to harbour *Francisella (F.) tularensis*. In Germany, Baden-Wuerttemberg represents an endemic focus for tularaemia. We have recently screened *Ixodes* and *Dermacentor* ticks from Baden-Wuerttemberg for presence of *F. tularensis*. 0.9 % of 916 *I. ricinus* ticks harboured this agent, while in 211 *D. marginatus* and *reticulatus* ticks *F. tularensis* was not detected. However, 50 % of these *Dermacentor* ticks carried *Francisella*-like endosymbionts (FLEs). Standard PCR protocols for identification of tularemia cannot discriminate between *F. tularensis* and FLEs.

To investigate the situation further, we screened 722 ticks of the genus *Dermacentor* from the archive of the Baden-Wuerttemberg State Health Office. 416 (57,6 %) of these ticks were positive for FLEs in the 16S rRNA real-time screening PCR as well as a PCR-ELISA of the *lpnA* gene. Results were confirmed by sequence analysis of the *lpnA* and mt16S rRNA gene of the ticks. In none of the *Dermacentor* ticks *F. tularensis* was detected.

Our results may suggest that in Southern Germany *F. tularensis* shows a tropism for *I. ricinus* ticks.

F 06

Serotype- and host-specific colonization of *Yersinia enterocolitica*

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Keywords: Yersinia enterocolitica, minipig colonization model, serotype- and host-specificity

The food-borne enteropathogen *Yersinia enterocolitica* is responsible for up to 6 000 – 7 000 cases of gastrointestinal diseases in Germany per year.
Enteropathogenic Yersiniae are able to colonize different host organisms which leads to several symptoms or severities of disease. While humans develop gut-associated as well as autoimmune diseases, pigs remain clinically healthy although the porcine intestinal tract can be efficiently colonized by *Y. enterocolitica.* Slaughtered pigs are known to be the most important reservoir of virulent enteropathogenic Yersiniae.
To study the dissemination of *Y. enterocolitica* in pigs, we established a minipig colonization model. 6-8 week old minipigs were infected with *Y. enterocolitica* strains of different serotypes and the bacterial burden of different organs was determined. We could show that *Y. enterocolitica* O:3 efficiently colonizes the porcine intestinal tract and is better adapted to pigs than serotype O:8 that is not able to survive inside the porcine intestinal tract. Based on the results of coinfection experiments with O:3 wildtype and different mutants we concluded that the O:3 specific factors $RovA_{S98}$ and IS*1667* contribute to the adaptation of this serotype to pigs. Additionally we performed all experiments also in mice to analyse a possible host-specificity of the different *Y. enterocolitica* serotypes. And indeed, we found out that the strains of the serogroups O:3 and O:8 are clearly adapted to different host organisms.

F 07

Population structure of ST398 based on mutation discovery

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Keywords: ST398, Population Structure, SNPs

Methicillin-resistant Staphylococcus aureus (MRSA) is a significant nosocomial and community acquired pathogen worldwide. In recent years sequence type 398 (ST398) was described as livestock associated MRSA. Nevertheless, latest studies have reported colonization and infection of companion animals and humans with ST398.

In this study the population structure of ST398 was investigated based on mutation discovery in 195 isolates. Isolates were collected from different hosts (human, pigs, poultry, horses, and cattle), and countries (Germany, Holland, USA, UK, Canada, Belgium, Italy, Denmark, Austria and Thailand). Spa type and SCCmec elements were determined for all isolates. Afterwards, denaturing high-performance liquid chromatography (dHPLC) was used for single-nucleotide polymorphism (SNP) analysis at approximately 100 loci (46 kb). Subsequently, polymorphic PCR products were sequenced and a minimum spanning tree (MST) was constructed by Bionumerics 6.5.

The correlation between the isolates' phylogeny and their geographic origin was not significant. The association of spa typing results with SNP-based phylogeny was weak. However, particular spa types were more dominant in certain countries. Interestingly, one clade within ST398 contained mostly isolates from horses, due to either epidemiological linkage or unknown host specificity factors.

F 08

Characterisation of pulmonary disorders induced by *Chlamydia psittaci* infection (bovine model)

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Keywords: Chlamydia psittaci, lung function, large animal model

Chlamydia psittaci (*Cp*) is a zoonotic respiratory pathogen. This prospective controlled study aimed at characterising *Cp* induced pulmonary dysfunctions by utilising a recently introduced calf model (Reinhold et al. 2012).

Each of 36 calves was inoculated intrabronchially, either with *Cp* (n=18) or with uninfected cell culture (n=18, controls). Non-invasive pulmonary function testing (impulse oscillometry, capnography, helium rebreathing technique) were applied to spontaneously breathing animals from 7 days before to 14 days post inoculation (dpi).

Compared to controls, both obstructive and restrictive pulmonary disorders were induced in calves exposed to *Cp*.

While disorders in respiratory mechanics lasted for 8-11 days, the pattern of spontaneous breathing was mainly altered in the period of acute illness (until 4 dpi). Here, expiration was more impaired than inspiration resulting in elevated functional residual capacity. Pulmonary ventilation per breath was characterised by a reduction in tidal volume (-25%) combined with an increased percentage of dead space volume. Although alveolar volume per breath was significantly reduced by 10%, hyperventilation occurred due to a compensatory doubling of respiratory rate resulting in significantly increased minute ventilation (+50%).

Pulmonary dysfunctions assessed in this bovine model provide relevant insights into the pathogenesis and pathophysiology of acute respiratory illness induced by *Cp* in the mammalian lung.

F 09

Cell free synthesis of *Vibrio parahaemolyticus* hemolysins

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Keywords: Toxins, protein expression

Vibrio parahaemolyticus is an important enteropathogenic bacteria which can cause diarrhea in humans. Infections occur mainly through uptake of raw or undercooked seafood and the bacteria are in some countries the leading cause of foodborne enteric diseases.
Major virulence determinants of *V. parahaemolyticus* are toxins that form pores in eukaryotic cells. The production of the thermostable direct hemolysin (TDH) is a characteristic of pathogenic *V. parahemolyticus* strains. All clinical strains isolated so far harboured the genes for TDH and/or TRH (TDH related hemolysin) and distinguish them from environmental strains of this *Vibrio* species. The hemolysins are produced as preproteins which are secreted through the bacterial cell wall by cleaving off a signal sequence and assemble into active toxins by forming tetramers. To establish detection systems for hemolysins we expressed the hemolysins in cell free systems to obtain pure toxins. For the purification of toxins affinity tags were added to facilitate isolation from the cell lysates. Addition of N-terminal Strep-tags and C-terminal His-tags was achieved by performing PCR with primers containing gene specific and tag specific sequences. For the expression in cell free systems linear templates were added directly to prokaryotic cell extracts. The functionality of newly synthesized toxins was tested by qualitative and quantitative hemolysis assays. In case of TDH the protein yield was in the range of 100 – 200 µg per mL. The purified toxins will be used for functionality tests and for the generation of antibodies which will be used for toxin detection in food samples or for the differential diagnostic of pathogenic and environmental strains.

F 10

Stability of genotyping targets of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) upon cultivation on different media and passage *in vitro* and *in vivo*

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Keywords: Mycobacterium avium subsp. paratuberculosis, genotyping, stability

MAP - the causative agent of paratuberculosis - affects domestic and wild ruminants worldwide. We recently suggested a combination of different typing techniques to yield a sufficient discriminatory power for exhaustive epidemiological studies.
In order to assess the reliability of this approach the stability of defined MAP genotypes were investigated after: (A) subcultivation on HEYM, Para-Löwenstein-Jensen- [PLJ], Para-Stonebrink-, Middlebrook-, and Watson-Reid-Medium [WR], (B) host passage within an animal model, and (C) 12 *in vitro* passages in PLJ. Field and reference strains as well as inoculation strain reisolated from five goats were genotyped by VNTR-, SSR-, and IS*900*-RFLP-analysis.
The number of tandem repeats [TR] at VNTR loci 292, X3, 25, 47, 7, and SSR loci 8 and 9 were stable. After cultivation in WR the number of repeated mononucleotides (>10) at SSR locus 1 or 2 of two strains was changed. More than 11 TR mononucleotides could not reliably be analysed because of polymorphisms within the target sequences or technical limitations. Three of 12 strains showed changed RFLP patterns after *in vitro* cultivation in different media, in contrast to the stable RFLP patterns after animal and 12 *in vitro* passages. As a consequence MAP isolates should not be frequently subcultivated before genotyping by IS*900*-RFLP.
When keeping in mind above-mentioned limitations for RFLP- and SSR-genotyping, the investigated techniques are reliably capable of differentiating MAP strains.

F 11

Investigations of virulence genes, adhesion and invasion of *Arcobacter butzleri* strains

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Arcobacter spp. has been assigned as a potential pathogen for human health and has been isolated from water and foods of animal origin. They differ from the related *Campylobacter* by their ability to grow at temperatures below 30°C and under aerobic and microaerobic conditions.
The presence of 10 putative virulence genes was determined in *A. butzleri* food/-environmental and human isolates by PCR. The genes *cia*B, *mvi*N, *pld*A, *tly*A, *cj*1349 and *cad*F were detected in 100%, *irg*A in 15.4%, *iro*E in 58.8%, *hec*B in 45.1% and *hec*A in 11.8% of the investigated 51 isolates. The adhesive and invasive abilities of 3 human and 3 chicken *A. butzleri* isolates were tested using the HT-29 cell line. The human and chicken isolates possessing all 10 virulence genes as well as the chicken isolate missing the genes *irg*A, *hec*A and *hec*B and the human isolate additionally missing *iro*E were adhesive. Of these adhesive isolates both isolates with all virulence genes as well as the chicken isolate missing the genes *irg*A, *hec*A and *hec*B were also invasive to HT-29 cells. To our knowledge the invasion ability of *A. butzleri* strains was described for the first time by our *in vitro* study.
In conclusion, the adhesive and invasive properties of strains isolated from food has been shown, therefore a human infection by consuming contaminated food might be possible.

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Comparison of LuxS sequences from *Campylobacter* spp. and influence of food matrices on AI-2 activity

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Keywords: Campylobacter, luxS

Numerous bacteria communicate via the small interspecies-specific signalling molecule autoinducer-2 (AI-2) generated via LuxS. Many bacterial species use communication (Quorum sensing) modulating physiological functions and expression of virulence factors. However, little is known about AI-2 activity in *Campylobacter* species.
The aim of this study was to compare LuxS amino acid (aa) sequence from *C. jejuni* and *C. coli* field strains and other *Campylobacter* species. The LuxS aa-sequences within strains of *C. jejuni* species and *C. coli* respectively showed vigorous conservation. The highest LuxS aa-homology was found among strains of *C. jejuni*, *C. coli* and *C. upsaliensis*. Correlation between this set of strains, *Arcobacter* spp. and *Vibrio* (*V.*) *harveyi* strains was higher as compared to other *Campylobacter* species analysed. All *Campylobacter* strains investigated that encoded *luxS* showed induction of bioluminescence in reporter strain *V. harveyi* BB170, however *C. lari* DSM 11375, *C. peloridis* and *C. insulaenigrae* showed no AI-2 activity. Since *Campylobacter* is the most important food borne zoonotic pathogen, AI-2 activity of *C. jejuni* NCTC 11168 and *V. harveyi* BB152 in milk and chicken juice was analysed. Cultivation in both matrices reduced AI-2 activity. This reduction was not a consequence of lower *luxS* gene expression. This occurs rather by a direct interaction of components of chicken juice with AI-2 molecules or with *V. harveyi* BB170 strain.

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Characterization of the protein-protein interaction of ferredoxin and lipoate synthase from *Toxoplasma gondii*

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Keywords: Toxoplasma gondii, ferredoxin, lipoate synthase

Toxoplasma gondii, the causative agent of toxoplasmosis, possess a unique plastid-derived organelle called apicoplast. The only known redox system therein consists of ferredoxin-$NADP^+$-reductase and its redox partner, ferredoxin (Fd). Fd donates electrons to different essential metabolic pathways in the apicoplast like to the last two enzymes of the isoprenoid precursor biosynthesis. Another important apicoplast-localized pathway is lipoic acid synthesis, whereby lipoate synthase (LipA) presumably receives electrons from Fd. To study this aspect recombinant TgLipA was purified and characterized. Like the *E. coli* protein TgLipA was shown to be a homodimer, both by a yeast two-hybrid approach and by gel filtration chromatography. Since electron transfer would require direct protein-protein interaction (PPI) between TgLipA and TgFd this was studied using two different two-hybrid systems, one eukaryotic (yeast two-hybrid) and one prokaryotic (*E. coli* reverse two-hybrid). The level of PPI was quantified by respective reporter assays, and in both cases significant reporter gene expression compared to respective controls could be observed, indicating that both proteins indeed physically interact. The bacterial reverse two-hybrid system will be used in combination with a genetically encoded cyclic peptide library in *E. coli* to screen in situ for PPI dissociators of the TgFd-TgLipA interaction, with the aim to define inhibitory pharmacophores for this essential redox system.

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