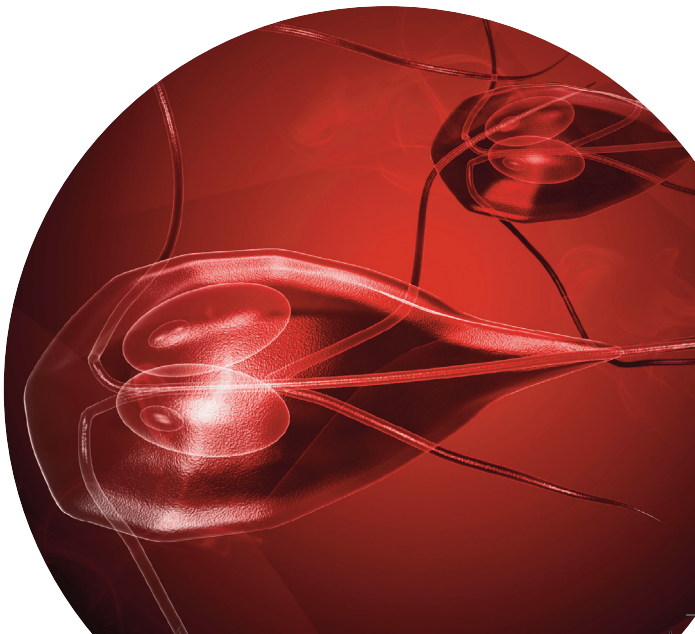
Nationale
Forschungsplattform
für Zoonosen

National Symposium on Zoonoses Research

6 - 7 October 2011 | Berlin

2011

Programme and Abstracts



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Nationale
Forschungsplattform
für Zoonosen

Editor
National Research Platform for Zoonoses

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Federal Research Institute for Animal Health
Institute of Novel and Emerging Infectious Diseases (INNT)
Südufer 10, 17493 Greifswald – Insel Riems, Germany

Table of Contents

Programme

Thursday 6 October, 2011

(Updated 19 September, 2011)

09.00 - 16.30 **Registration**

11.00 – 12.30 Plenary Session (Room Ballsaal)

11.00 – 11.30 **Opening Ceremony and Welcome Notes**

Stephan Ludwig (National Research Platform for Zoonoses), Angela Lindner (BMBF), Karin Schwabenbauer (BMELV) und Kirsten Reinhard (BMG)

11.30 – 12.00 **Global surveillance, early warning and control of emerging diseases: an increasingly coordinated activity still full of surprises**
Stephane de la Rocque, Rome, Italy

12.00 – 12.30 **An ecohealth perspective on highly pathogenic avian influenza H5N1 in South-East Asia**
Dirk Pfeiffer, London, UK

12.30 – 14.00 **Lunch Break**

14.00 – 15.30 Session Innate and adaptive immune response (Room Ballsaal)
Chairs: Ralph Goethe and Eberhard Straube

Biological relevance and mechanism of interferon escape by lyssavirus phosphoproteins
M. Rieder, L. Drechsel, K.-K. Conzelmann

HPAIV H5N1 virus escaped from neutralising immune pressure by unusual variation of the neuraminidase encoding segment
D. Kalthoff, S. Röhrs, D. Höper, B. Hoffmann, M. Beer

A new role of the complement system in the interaction with intracellular bacteria: Complement drastically increases survival in *Chlamydia psittaci* lung infection
J. Ebeling, P. Dutow, K. Sommer, K. Janik, S. Glage, B. Tümmler, A. Munder, R. Wetsel, K. Sachse, A. Klos

Novel murine infection models provide deep insights into the „Ménage à Trois" of *Campylobacter jejuni*, microbiota and host innate immunity
S. Bereswill, A. Fischer, R. Plickert, L.-M. Haag, B. Otto, A. A. Kühl, J. I. Dashti, A. E. Zautner, M. Muñoz, C. Loddenkemper, U. Groß, U. B. Göbel, M. M. Heimesaat

Magic compounds: ingredients of red wine, curry wurst and cholesterol-lowering drugs ameliorate acute small intestinal inflammation in mice following oral *Toxoplasma gondii* infection
M. M. Heimesaat, M. Muñoz, A. Fischer, R. Plickert, L.-M. Haag, B. Otto, A. A. Kühl, C. Loddenkemper, U. B. Göbel, S. Bereswill

Cellular production of pro-inflammatory cytokines (IL-17, IL-19) and chemokines (CCL24/MCP-4) in alveolar echinococcosis patients at distinct states of infection
C. J. Lechner, B. Grüner, P. Kern, P. T. Soboslay

14.00 – 15.30 Session Methods and diagnostics (Room Lankwitz)
Chair: Dag Harmsen

***Gaussia princeps* luciferase (Gluc) can be used to monitor type I protein secretion in *Salmonella enterica* serovar Typhimurium**
T. Wille, R. G. Gerlach

Peptide-microarray analysis of in-silico predicted epitopes for serological diagnosis of *Toxoplasma gondii* in infected humans
P. Maksimov, J. Zerweck, A. Maksimov, A. Hruzik, U. Groß, K. Spekker, W. Däubener, S. Werdermann, O. Niederstrasser, E. Petri, M. Mertens, R. G. Ulrich, F. J. Conraths, G. Schares

Rapid and sensitive detection of Orthopoxviruses by an Abicap-Immunofiltration column
D. Stern, M. Zydek, M. Pietraszczyk, D. Pauly, B. Dorner, F. Lisdat, P. Miethe, A. Nitsche

Novel serological and molecular diagnostic tools for Rift Valley Fever Virus detection and surveillance
S. Jäckel, M. Eiden, A. Balkema-Buschmann, M. Dauber, H. Unger, M. H. Groschup

A short conserved linear epitope of influenza hemagglutinin H5 identified by recombinant antibody generation
J. Mersmann, D. Meier, A. Postel, C. Grund, M. Rohde, T. Harder, S. Dübel

Evaluation of antiviral activity of novel antiseptic peptides against influenza viruses
J. Richter, K. Brandenburg, G. Gabriel

14.00 – 15.30 Session Pathogen-cell interaction I (Room Steglitz)
Chair: Frank T. Hufert

Increased circulating endothelial progenitor cells (cEPC) in patients infected with Puumala hantavirus
E. Krautkrämer, S. Grouls, N. Rafat, D. Hettwer, M. Zeier

Puumala virus NSsORF supports replication in an Arvicolinae-derived reservoir host cell line
R. Franke, R. Ulrich, R. Riebe, M. Lenk, D. H. Krüger, A. Rang

The nonstructural protein 3 papain-like protease of a Bat-Coronavirus inhibits interferon induction and promotes viral replication in human cells
D. Niemeyer, E. Jentgens, C. Drosten, M. A. Müller

Comparative analysis of the Ebola virus glycoprotein interactions in potential reservoir bat cells and human cells
A. Kühl, M. Hoffmann, M. A. Müller, V. J. Munster, K. Gnirß, M. Kiene, T. S. Tsegaye, G. Behrens, G. Herrler, H. Feldmann, C. Drosten, S. Pöhlmann

The open reading frame 8 of SARS-Coronavirus displays host adaptation and has an influence on virus replication in primate and bat cell culture
D. Muth, J. F. Drexler, F. Gloza-Rausch, T. Binger, H. Roth, S. Pfefferle, K. Zimmermann, A. Pfeifer, C. Drosten, M. A. Müller

Comparison of the intracellular distribution of lyssavirus matrix proteins
R. Pollin, S. Finke

15.30 - 16.30 Poster Exhibition

16.30 – 17.30	**Plenary Session (Room Ballsaal)**
16.30 – 17.00	**Pandemic H1N1 and beyond** Jürgen Richt, Manhattan Kansas, USA
17.00 – 17.30	**Diversity, impact and control of chlamydial infections in ruminants** David Longbottom, Penicuik, UK
17.30 – 18.00	**Informed-consent templates for the sampling of animals – presentation and discussion (in German)** Jürgen W. Goebel, Bad Homburg v.d. Höhe
18.00 – 19.30	**General Assembly National Research Platform for Zoonoses (in German)** - Annual report - Election of the internal advisory board S. C. Semler, S. Ludwig, M. H. Groschup (National Research Platform for Zoonoses)
from 19:30	**Get-together**

Programme - Friday 7 October, 2011

09.00 – 10.00 **Session Epidemiology, surveillance and risk assessment I**
(Room Ballsaal)
Chairs: Dirk Schlüter and Lothar Wieler

Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from poultry meat
A. T. Feßler, K. Kadlec, M. Hassel, T. Hauschild, C. Eidam, R. Ehricht, S. Monecke, S. Schwarz

Comparative genetic analysis of canine and human *S. aureus*-isolates reveals a substantial proportion of extended host spectrum genotypes (EHSG)
S. Vincze, A. Lübke- Becker, S. Monecke, T. Semmler, I. Stamm, P. A. Kopp, L. H. Wieler, B. Walther

Adherent-invasive *Escherichia coli* (AIEC) – a group of pathogens associated with Crohn's disease in humans – are frequently isolated from enteritis in cats, dogs, and swine
M. Martinez-Medina, J. Garcia-Gil, N. Barnich, L. H. Wieler, C. Ewers

Genetically different *T. gondii*, isolated from a single feline faecal sample from Germany, have different virulences in BALB/c mice.
D. C. Herrmann, A. Bärwald, N. Pantchev, M. G. Vrhovec, F. J. Conraths, G.Schares

09.00 – 10.00 Session Wildlife and new or re-emerging zoonoses I
(Room Steglitz)
Chair: Sandra Eßbauer

Emerging infectious diseases: impact of anthropogenic change in land use on prevalence of blood parasites in Neotropical bats
V. M. Cottontail, E. K. V. Kalko

Henipavirus RNA in bats from bushmeat markets
S. Weiss, K. Nowak, J.-V. Mombouli, F. H. Leendertz

Bat and canine adenoviruses: possible ancient inter-species transmission?
C. Kohl, B. Harrach, K. Mühldorfer , G. Wibbelt, A. Nitsche, A. Kurth

Bat rabies in Germany - discovery of a novel lyssavirus in a Natterer's bat (*Myotis nattereri*)
T. Müller, B. Hoffmann, D. Höper, M. Beer, J. Teifke, S. Finke, T. C. Mettenleiter, C. Freuling

09.00 – 10.00 Session Pathogen-cell interaction II
(Room Lankwitz)
Chair: Martin Beer

A single point mutation (Y89F) within the non-structural protein 1 of influenza A viruses dramatically limits lung epithelial cell tropism and virulence in mice.
E. R. Hrincius, L. Gensler, A.-K. Henneke, D. Anhlan, J. McCullers, S. Ludwig, C. Ehrhardt

Biphasic action of p38 MAPK signaling in the influenza A virus induced primary and secondary host gene response
Y. Börgeling, M. Schmolke, D. Viemann, J. Roth, S. Ludwig

Adaptive mutations in NEP can compensate defects in RNA replication of avian Influenza viruses
B. Mänz, L. Brunotte, M. Schwemmle

Targeting of the TRIM25-RIG-I axis by the Influenza B NS1 protein
M. Budt, D. Voß, R. Davina Nunez, T. Wolff

10.00 - 11.00 Poster Exhibition

11.00 – 12.30 Session Epidemiology, surveillance and risk assessment II (Room Lankwitz)
Chair: Christian Drosten

A high prevalence of HEV-specific antibodies observed in slaughterers may raise the question of HEV transmission during the process of slaughtering
A. Krumbholz, U. Mohn, J. Lange, M. Motz, J. Wenzel, W. Jilg, M. Walther, E. Straube, P. Wutzler, R. Zell

Rats as potential reservoir for cowpox virus infections
A. Puppe, C. Zübert, A. Nitsche, A. Kurth

In search for factors driving hantaviruses and rickettsia – studies in an climate-altitude gradient
S. Eßbauer, B. Thoma, A. Osterberg, P. Bleichert, S. Schex, S. Speck, G. Dobler, C. Bässler, J. Müller

Zoonotic behavior of the pandemic swine influenza virus in Switzerland
S. Heidemeyer, M. Engels, E. Loepfe, C. Wunderwald, M. Ackermann

Phylogenetic analysis of yellow fever virus isolates from West Africa
N. Stock, H. Laraway, M. Niedrig, A. Sall

Molecular epidemiology of rabies in the Middle East and Central Asia with special emphasis on Iraq and Afghanistan
C. M. Freuling, L. Fuhrmann, O. Aylan, H. Un, A. R. Fooks, L. McElhinney, N. Johnson, T. Müller

11.00 – 12.30 Session Wildlife and new or re-emerging zoonoses II
(Room Steglitz)
Chair: Gudrun Wibbelt

Discovery of polyomaviruses in non-human primates: indicators for the existence of unknown human polyomaviruses
N. Scuda, S. Calvignac-Spencer, C. Boesch, F. H. Leendertz, B. Ehlers

Infection studies with West Nile virus lineage 1 and 2 in large falcons
J. Angenvoort, U. Ziegler, D. Fischer, C. Fast, M. Eiden, S. Revilla-Fernández, N. Nowotny, J. Garcia de la Fuente, M. Lierz, M. H. Groschup

Characterization of an emerging Mongolian TBE virus strain
S. Frey, I. Mossbrugger, S. Speck, D. Rendoo, D. Altantuul, J. Battsetseg, D. Otgonbaatar, D. Tserennorov, L. Zöller, R. Wölfel, S. Eßbauer, G. Dobler

The German arbovirus surveillance and mosquito monitoring program, 2009 - 2010
H. Jöst, N. Becker, E. Tannich, S. Günther, J. Schmidt-Chanasit

Interdisciplinary zoonosis research in Germany: the network "Rodent-borne pathogens"
R. G. Ulrich

Multiresistant *Escherichia coli* from brown rats: phylogenetic background and virulence potential
S. Guenther, A. Bethe, M. Grobbel, R. G. Ulrich, L. H. Wieler, C. Ewers

11.00 – 12.30 Session Pathogen-cell interaction III (Room Ballsaal)
Chairs: Christian Menge and Konrad Sachse

Identification of glycolipid receptors of virulence factors from zoonotic pathogens
J. Müthing

Synergistic adaptive mutations in the HA and PA lead to increased virulence of pandemic 2009 H1N1 influenza A virus in mice.
R. Seyer, E. R. Hrincius, D. Ritzel, M. Abt, H. Marjuki, J. Kühn, T. Wolff, S. Ludwig, C. Ehrhardt

Analyze the adaptation of influenza viruses by using well-differentiated porcine airway epithelial cells
D. Punyadarsaniya, I. Hennig-Pauka, C. Winter, C. Schwegmann-Wessels, G. Herrler

Mouse model of respiratory orthopoxvirus infection to study virulence factors essential in the development of fatal disease
G. Sutter, A. Volz, M. Gratz, Y. Suezer, M. Majzoub, K. Hanschmann, A. Schwantes, U. Kalinke, M. Lehmann, M. Kremer

Type III-secreted protein IncA of *Chlamydia psittaci* interacts with host cell protein G3BP1 to reduce c-Myc protein concentration during the chlamydial infection cycle
N. Borth, K. Litsche, C. Franke, K. Sachse, H. P. Saluz, F. Hänel

The function of the HCN-domain of clostridial neurotoxins
J. Strotmeier, J. Deppe, S. Mahrhold, T. Binz, H. Bigalke, A. Rummel

11.00 – 12.30 Career Session (in German) (Room Zehlendorf)

Faszination zoonotische Forschung – von der Uni ans Forschungsinstitut
Petra Dersch, Braunschweig

Zoonosenbekämpfung – eine Herausforderung für Wissenschaft und Institutionen
Karin Schwabenbauer,Bonn

Wege in die Translationsforschung
Ulrich Kalinke, Hannover

Akademische Laufbahn: Plan B
Ulrike Gerischer, Göttingen

Diskussion

12.30 – 14.00 Lunch Break

14.00 – 16.30 Plenary Session (Room Ballsaal)

14.00 – 14.30 **Preventing influenza: research and regulatory aspects**
Ralf Wagner, Langen

14.30 – 15.00 **Zoonoses research in Germany – quo vadis?**
Thomas C. Mettenleiter, Greifswald - Insel Riems

15.00 – 15.45 **EHEC outbreak 2011 in Germany – an overview**

Phenotypic and genotypic traits of EHEC O104:H4 isolates
Helge Karch, Münster

	Clinical manifestation and disease management: Experiences from the STEC O104:H4 outbreak in Hamburg Jacob Cramer, Hamburg
15.45 – 15.50	**DFG Priority Programme "Ecology and species barriers in emerging viral diseases" at a glance** Christian Drosten, Bonn
15.50 – 16.15	**Poster Awards**
16.15	**Closing Ceremony**

General information

Date and venue
6 – 7 October 2011
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)

Participating scientific associations
German Society of Hygiene and Microbiology (DGHM), www.dghm.org

German Veterinary Medical Society (DVG), www.dvg.net

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 2010-2011.

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

Language
The official conference language is English.

Continuous medical education
The National Symposium on Zoonoses Research 2011 is registered for 12 CME points of category B by the Berlin Chamber of Physicians (Ärztekammer Berlin) as follows:

Thursday, 6 October, 2011	11.00 – 12.30 am	3 points
Thursday, 6 October, 2011	2.00 – 6.00 pm	3 points
Friday, 7 October, 2011	9.00 – 12.30 am	3 points
Friday, 7 October, 2011	2.00 – 5.30 pm	3 points

Please note, that you need four barcode labels. It is necessary to sign in the list twice a day.

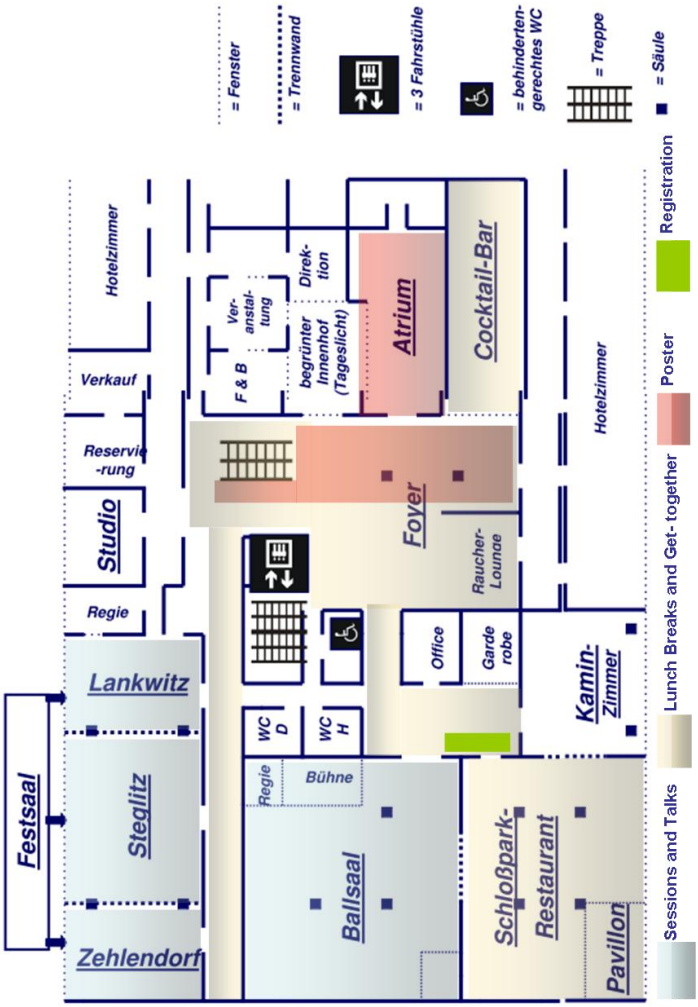
Continuous veterinary education
The National Symposium on Zoonoses Research 2011 is registered for 9 hours (ATF-Stunden) by the Federal Chamber of Veterinarians (Bundestierärztekammer).

Oral presentations
Oral presentations should be handed over on a common data carrier at the registration desk on Thursday, 6th 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 flour. WLAN will be provided without charge.

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

Floor plan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Hotelzimmer
Verkauf
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Studio
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WC D
WC H
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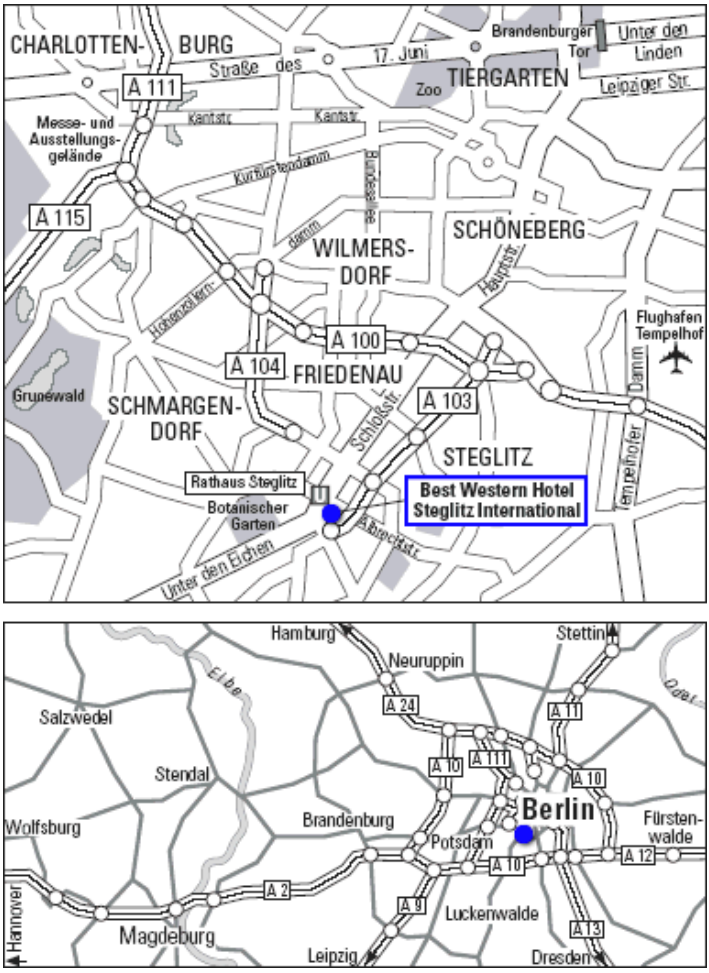
Bühne
Ballsaal
F & B
Ver-anstal-tung
begrünter Innenhof (Tageslicht)
Direk-tion
Atrium
Cocktail-Bar
Foyer
Raucher-Lounge
Office
Garde robe
Kamin-Zimmer
Hotelzimmer
Schloßpark-Restaurant
Pavillon
Sessions and Talks
Lunch Breaks and Get- together
Poster
Registration

Site plan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Kantstr.
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Kurfürstendamm
Bundesallee
A 115
WILMERS-
DORF
SCHÖNEBERG
Hauptstr.
Hohenzollern-
damm
A 100
A 104
FRIEDENAU
A 103
Flughafen Tempelhof
Damm
Grunewald
SCHMARGEN-
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Schloßstr.
STEGLITZ
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U
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Best Western Hotel
Steglitz International
Albrechtstr.
Unter den Eichen
Tempelhofer
Hamburg
Neuruppin
Stettin
Elbe
Oder
A 24
A 11
Salzwedel
A 10
A 111
A 10
Stendal
Berlin
Wolfsburg
Brandenburg
Potsdam
Fürsten-
walde
A 10
A 12
A 2
A 9
Luckenwalde
A 13
Hannover
Magdeburg
Leipzig
Dresden

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 2011.

The programme of our meeting represents again the broad spectrum of zoonoses research. Research groups from all over Germany will present their studies concerning pathogenesis and immunity, epidemiology and risk assessment, wildlife and (re-) emerging zoonoses, genomics and diagnostics. Many thanks to all, who submitted abstracts for oral or poster presentations! Renowned speakers complete the programme and will give plenary talks to current issues.

This year was determined by the EHEC-outbreak in May and June in Germany. Therefore we are very proud to welcome Helge Karch and Jakob Cramer who will join our symposium and refer on the outbreak.

We would like to encourage all participants to pay close attention to the poster sessions. These sessions offer the opportunity to initiate the scientific information exchange and foster interdisciplinary collaborations on top level. The best three posters of young researchers will be honored by our poster award.

Once again we offer a career-session to give young researchers the opportunity to exchange opinions concerning different career pathways.

We greatly look forward to welcoming you in Berlin. Please enjoy the scientific programme and your stay.

S. Ludwig (Münster) M. H. Groschup (Greifswald – Insel Riems) S. C. Semler (Berlin)
Directors of the National Research Platform for Zoonoses

Welcome Notes of the Federal Ministries

Grußwort des Bundesministeriums für Bildung und Forschung zum Nationalen Symposium für Zoonosenforschung 2011

SARS, Schweinegrippe und jetzt der EHEC-Erreger: Die Gefahr des Auftretens alter und auch neuer Erreger erfordert eine krankheitsübergreifende Erforschung und inhaltlich wie methodisch breite wissenschaftliche Ansätze. Deshalb ist und bleibt die Förderung der Zoonosenforschung in Deutschland ein wichtiger Bestandteil des Aktionsfeldes Volkskrankheiten im neuen „Rahmenprogramm Gesundheitsforschung" der Bundesregierung.

Um aktuellen Entwicklungen im Bereich der Forschung zu Zoonosen gerecht zu werden, wurde die Bekanntmachung der neun geförderten Zoonosen-Verbünde für zwei neue Themenfelder für eine zweite Förderphase geöffnet. Zum einen konnten Verbundanträge zu Bakterien mit Antibiotika-Resistenzen, die vom Tier auf den Menschen übertragen werden, eingereicht werden. Damit wird ein Beitrag zur Umsetzung der Deutschen Antibiotika-Resistenzstrategie (DART) der Bundesministerien für Bildung und Forschung (BMBF), für Gesundheit (BMG) und für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) geleistet. Zum anderen wurde die Bekanntmachung geöffnet für Verbundanträge zu vernachlässigten zoonotischen Infektionskrankheiten (NZDs) in ärmeren Ländern. Ich freue mich, dass wir seit November 2010 zu jedem dieser beiden Themenfelder je zwei neue Verbünde fördern und somit den Kreis der geförderten Zoonosenforscherinnen und -forscher in Deutschland erweitern.

Krankheiten wie Dengue-Fieber, Schlafkrankheit oder bakterielle Pneumonie, unter denen vor allem die Ärmsten der Welt leiden, stehen nicht immer im Mittelpunkt des Forschungsinteresses der Industrienationen, da sie für die hochentwickelten Länder weniger relevant sind. Stellvertretend für Deutschland stellt sich das Bundesforschungsministerium dieser Verantwortung und investiert verstärkt in die Erforschung von vernachlässigten und armutsassoziierten Krankheiten. So werden neben den beiden o.g. Verbünden seit 2010 drei Nachwuchsgruppen gefördert, die sich der Erforschung parasitärer Wurmerkrankungen (bspw. Schistosomen) widmen.

Anfang Mai dieses Jahres haben wir unser Förderkonzept gegen vernachlässigte und armutsassoziierte Krankheiten vorgestellt. Ein Element des neuen Förderkonzepts ist die Unterstützung von Produktentwicklungspartnerschaften, kurz PDP. Hiermit wollen wir einen neuen Weg internationaler Kooperationen gehen und gezielt die Entwicklung kostengünstiger Präventionsmethoden, Diagnostika und Medikamente fördern. Die Förderung der PDP zielt auf zwei Krankheitsgruppen: 1 - die von der WHO definierten 17 vernachlässigten tropischen Krankheiten wie Leishmaniose, Dengue-Fieber oder Tollwut sowie 2 - auf Krankheiten, die zu hoher Mortalität bei Kindern und Schwangeren in Entwicklungsländern führen wie bakterielle Pneumonie, Meningitis, Durchfallerkrankungen, rheumatisches Fieber oder Malaria.

Ich freue mich, dass das Treffen der Zoonosenforscherinnen und –forscher, das in diesem Jahr zum fünften Mal stattfindet, inzwischen zu einer festen Größe in der Forschungslandschaft geworden ist. Die Organisation des Symposiums übernimmt auch in diesem Jahr die Geschäftsstelle der Nationalen Forschungsplattform für Zoonosen.

Ich wünsche allen Beteiligten eine erfolgreiche Tagung und anregende Diskussionen in Berlin.

Dr. Angela Lindner,
Leiterin des Referats Gesundheitsforschung
Bundesministerium für Bildung und Forschung

Grußwort des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz

Seit dem massiven Auftreten der Hochpathogenen Aviären Influenza 2003 in Südostasien, die nach wie vor Opfer bei Mensch und Tier fordert, ist es unumstritten, dass zum Schutz der Bevölkerung vor Zoonosenerregern gezielte interdisziplinäre Forschung notwendig ist. Hierzu hat sich die Bundesregierung früh bekannt, und BMG, BMELV und BMBF fördern seit einigen Jahren auf diesem Gebiet gemeinsame Forschungsaktivitäten.

Für BMELV haben Zoonosen eine ganz besondere Bedeutung, da es nicht nur für die Tiergesundheit, sondern auch für die Sicherheit der Lebensmittel tierischen Ursprungs zuständig ist. Darüber hinaus vertritt BMELV Deutschland beim Codex Alimentarius und der Welttiergesundheitsorganisation, wo die Regelungen für den globalen Handel mit Tieren und tierischen Produkten beraten werden. Damit kommen die Bereiche zusammen, die für Entstehung und Ausbreitung von Zoonosen eine entscheidende Rolle spielen.

Ziel der Bemühungen des BMELV in seinem Zuständigkeitsbereich ist es daher nicht nur Auftreten und Verbreitung von Zoonosenerregern zu minimieren, sondern auch die Entstehung neuer Zoonosen zu vermeiden. Dazu benötigt es dringende Erkenntnisse aus der Forschung, um entsprechende Handlungsoptionen zu entwickeln.

Klimawandel, Globalisierung des Handels und Veränderungen der Agrar-Ökosysteme weltweit sind Faktoren, die die Entstehung neuer Zoonosen, bzw. das Auftreten exotischer Krankheiten in neuen Regionen begünstigen. Dies stellt die Gesundheitssysteme vor besondere Herausforderungen. Die jüngste durch enterohämorrhagische E.coli ausgelöste Epidemie in Deutschland hat dies sehr plastisch vor Augen geführt: erst durch die Zusammenführung der Erkenntnisse aus Forschung, Klinik und Lebensmittelüberwachung konnte die Ursache geklärt und die Epidemie gestoppt werden.

In Bezug auf Forschung wird das Engagement des BMELV auch an den erheblichen Investitionen beim Friedrich-Loeffler-Institut nicht nur am Standort Riems sondern auch in Jena deutlich. Dabei spielt die Zoonosenforschung eine große Rolle – schließlich ist das Institut eins der Zoonosen-Referenz-Zentren der Welttiergesundheitsorganisation!

Darüber hinaus sind dem BMELV die Forschungsplattform sowie die Forschungsverbünde, in denen offene Fragen interdisziplinär

bearbeitet werden, wichtige Ansprechpartner, die weiterhin gefördert werden müssen. Gleichzeitig sollten diese aber auch verstärkt international sichtbarer sein, nicht zuletzt um neue Partnerschaften zu entwickeln. An der international geführten Debatte zum „One Health"-Konzept sollten sie sich aktiv beteiligen. Hierzu bietet die diesjährige Tagung ein gutes Forum.
Ich wünsche der Tagung in diesem Sinne einen erfolgreichen Verlauf, der Plattform eine Stärkung ihres Netzwerks und den Teilnehmern viele gute Gespräche, aus denen neue kreative gemeinsame Forschungsansätze entwickelt werden können.

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

Grußwort des Bundesministeriums für Gesundheit

Infektionskrankheiten gehören nach Angaben der Weltgesundheitsorganisation WHO zu den weltweit häufigsten Todesursachen für Menschen. Sie machen ein Viertel aller Todesfälle aus. Etwa zwei Drittel aller Infektionskrankheiten sind so genannte Zoonosen und bei neu auftretenden Krankheiten liegt der Anteil der Zoonosen gar bei 75 Prozent.

Mit der Zoonosenplattform wurde ein institutionalisierter Zusammenschluss der Forschungsverbünde zu Zoonosen und Wissenschaftlern, die im Bereich Zoonosen in Deutschland forschen, geschaffen. Die Forschung im Bereich der zoonotischen Infektionskrankheiten erfordert die enge Zusammenarbeit von Human- und Veterinärmedizinern, sowohl in der Grundlagenforschung als auch bei der patientenorientierten klinischen Forschung.

Eines der wesentlichen Ziele der Nationalen Forschungsplattform für Zoonosen ist die Stärkung der Zusammenarbeit der Zoonosenforscher und die Vernetzung von Human- und Veterinärmedizin auf allen Ebenen. Um das Themenfeld der zoonotischen Infektionserkrankungen erfolgreich bearbeiten zu können, ist die Zusammenarbeit von Human- und Veterinärmedizin eine grundlegende Voraussetzung.

Mit dem EHEC-Ausbruch im Mai/Juni diesen Jahres in Deutschland sind die Zoonosen wieder verstärkt ins öffentliche Bewusstsein gerückt. Das diesjährige Symposium greift dieses aktuelle Geschehen auf.

Um zoonotische Infektionskrankheiten besser bekämpfen zu können, brauchen wir dringend ein besseres Verständnis des Übergangs der Erreger auf einen neuen Wirt und der für sein Überleben notwendigen Anpassungsvorgänge. Der Aufbau geeigneter Strukturen für die Zusammenarbeit kann erhebliche Synergien mobilisieren und Prävention, Diagnose und Therapie von zoonotischen Infektionskrankheiten langfristig verbessern.

Das Symposium widmete sich im letzten Jahr der Förderung und Beteiligung von Nachwuchsforschern. Diese Fokussierung leistete einen herausragenden Beitrag jungen Akademikern die Möglichkeit zu geben, ihre Forschungsergebnisse vor

internationalem Publikum präsentieren und diskutieren zu können.

Ich freue mich sehr, dass auch dieses Jahr der wissenschaftliche Nachwuchs wieder im Mittelpunkt des Symposiums steht und begrüße die Verstetigung der Schwerpunktsetzung auf junge Wissenschaftler beim Nationalen Symposium für Zoonosenforschung. Damit wird den Nachwuchsforschern auch im Jahr 2011 ausreichend Raum und Zeit gewidmet.

Das Nationale Symposium für Zoonosenforschung stößt national, aber auch international, auf großes Interesse. Gerade für die Zoonosenforschung ist es unabdingbar, nicht nur interdisziplinär, sondern auch international sich den Herausforderungen der übertragbaren Krankheiten zu stellen. Wir wissen alle, dass Zoonosen nicht an Landesgrenzen Halt machen und sie sich weltweit auf dem Vormarsch befinden. Von daher hat eine internationale Ausrichung der zoonotischen Forschung eine große Bedeutung.

Ich bin sicher, dass vom Nationalen Symposium für Zoonosenforschung viele neue Impulse für die weitere Arbeit ausgehen werden. Das jährlich stattfindende Symposium bietet die Gelegenheit, aktuellste Forschungsergebnisse disziplinübergreifend zu diskutieren.

Allen Teilnehmerinnen und Teilnehmern wünsche ich eine gelungene Veranstaltung und einen anregenden wissenschaftlichen Austausch zum Thema Zoonosenforschung.

Dr. Kirsten Reinhard
Bundesministerium für Gesundheit

Oral Presentations

Session Innate and adaptive immune response

6 October, 2011
14.00 – 15.30

Room Ballsaal
Chairs: Ralph Goethe and Eberhard Straube

Biological relevance and mechanism of interferon escape by lyssavirus phosphoproteins

M. Rieder, L. Drechsel, K.-K. Conzelmann

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Forschungsverbund Lyssaviren

Keywords: Lyssavirus, rabies, interferon, host range

The phosphoprotein P of the neurotropic rabies virus (RV) is a powerful antagonist of the host interferon response in human and murine cells preventing both RIG-I-like receptor-mediated IFN induction and IFN-mediated JAK/STAT signaling. In addition, P is an essential cofactor of the viral polymerase and is required for encapsidation of viral RNA. We have previously identified domains of P required for inhibition of IFN induction but not for the other functions of P. Rabies viruses lacking the critical P residues, like SAD ΔInd1, are completely apathogenic even after intracerebral injection into mouse brains, demonstrating that neurovirulence of IFN resistant RV correlates with the capacity to prevent activation of IRF3 (Rieder et al., JVI 2011). Notably, the P protein of a bat-adapted rabies-related virus, European bat lyssavirus type 1 (EBLV-1) was also found to interfere with IFN induction in human cells. Current experiments involving transient expression of P proteins and generation of chimeric viruses are aimed at comparing the mechanisms and the IFN-inhibitory capacity of RV and EBLV-1 P proteins, in order to reveal their contribution to the host range of lyssaviruses.

HPAIV H5N1 virus escaped from neutralising immune pressure by unusual variation of the neuraminidase encoding segment

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Keywords: HPAIV H5N1, immune escape, neuraminidase

Partial immunity normally leads to antigenic drift by selectively favouring viral escape mutants that probably already exist in the quasispecies representing the ancestor virus. To investigate this phenomenon, highly pathogenic avian influenza virus (HPAIV) of subtype H5N1 was serially passaged 50 times in embryonated-egg culture under the selective pressure of a neutralising, polyclonal chicken-derived antiserum. Full-length genome sequences of the resulting mutant as well as of a control virus passaged without serum pressure were determined by deep sequencing and comparison of the sequence data revealed major changes especially in the neuraminidase encoding segment of the selected virus mutant. Interestingly, the in vitro-generated novel HPAIV H5N1 virus comprised only half of the neuraminidase encoding segment with several frame shifts and only one open reading frame of about 50 amino acids. The sequence changes of this new influenza virus – named "H5N1del MaDo" - were associated with a slightly reduced in vitro growth, but a complete loss of virulence for chicken (intravenous pathogenicity index = 0). Nevertheless, the cleavage site of the hemagglutinin of "H5N1del MaDo" was unchanged and electron microscopy analysis did not show any unusual accumulation of virus particles at the cell surface.
The generated escape virus mutant is the first "neuraminidase-negative" influenza virus generated by immune-escape without any external neuraminidase supplement. Further in vitro and in vivo analysis of this unique influenza virus are performed and allow better insights into the function and role of the neuraminidase of influenza A viruses and its role for virus replication and pathogenesis.

A new role of the complement system in the interaction with intracellular bacteria: complement drastically increases survival in *Chlamydia psittaci* lung infection

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Keywords: *Chlamydia psittaci*, complement system, defence, C3

The complement system directs immune effector functions and modulates the intensity of innate and specific immune responses. It is activated by extracellular pathogens and damaged cells. This project demonstrates its important role in *C. psittaci* lung infection using several complement factor and receptor knock-out (or deficient) mice. Mice were intranasally infected with the non-avian strain DC 15 and sacrificed on days 4, 9, and 21 for analysis. Weight and clinical score were determined daily. Complement activation occurred before symptoms of pneumonia became apparent. Surprisingly, following a phase of partial protection, all complement factor $C3^{-/-}$ mice died until day 17 post infection. Compared to wildtype controls, the mice lacking most complement effector functions were 100 times more susceptible to the intracellular bacteria. During the phase of partial protection of the $C3^{-/-}$ mice until day 9, more granulocytes migrated into their inflamed lungs, proinflammatory cytokines were more elevated, Th1-polarization seemed to be even stronger. The bacterial load in the lung of the knock-out mice was significantly decreased, they lost less weight and their clinical score was better. Our data indicate that C3-effector functions are harmful early in *C. psittaci* infection; increased Chlamydia uptake mediated by C3b might be the reason for that. However, later in infection, when specific immunity becomes essential for defence, complement activation seems to be necessary for the fine-tuning of an otherwise deleterious immune response. Our data show for the first time the strong influence of complement activation on the outcome of an infection with intracellular bacteria.

Novel murine infection models provide deep insights into the „ménage à trois“ of *Campylobacter jejuni*, microbiota and host innate immunity

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Keywords: *Campylobacter jejuni*, colonization resistance, human microbiota, host-pathogen-interaction, bacterial formic acid metabolism

Although *Campylobacter jejuni*-infections have a high prevalence worldwide and represent a significant socioeconomic burden, it is still not well understood how *C. jejuni* causes intestinal inflammation. Detailed investigation of *C. jejuni*-mediated intestinal immunopathology is hampered by the lack of appropriate vertebrate models. In particular, mice display colonization resistance against this pathogen. To overcome these limitations we developed a novel *C. jejuni*-infection model using gnotobiotic mice in which the intestinal flora was eradicated by antibiotic treatment. These animals could then be permanently associated with a complete human (hfa) or murine (mfa) microbiota. After peroral infection *C. jejuni* colonized the gastrointestinal tract of gnotobiotic and hfa mice for six weeks whereas mfa mice cleared the pathogen within two days. Strikingly, stable *C. jejuni* colonization was accompanied by a pro-inflammatory immune response indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils and apoptotic cells as well as increased concentrations of TNF-α, IL-6, and MCP-1 in the colon mucosa of hfa mice. Analysis of MyD88$^{-/-}$, TRIF$^{-/-}$, TLR4$^{-/-}$, and TLR9$^{-/-}$ mice revealed that TLR4- and TLR9-signaling was essential for immunopathology following *C. jejuni*-infection. Interestingly, *C. jejuni*-mutant strains deficient in formic acid metabolism and perception induced less intestinal immunopathology compared to the parental strain infection. In summary, the murine gut flora is essential for colonization

resistance against *C. jejuni* and can be overcome by reconstitution of gnotobiotic mice with human flora. Detection of *C. jejuni*-LPS and -CpG-DNA by host TLR4 and TLR9, respectively, plays a key role in immunopathology. Finally, the host immune response is tightly coupled to bacterial formic acid metabolism and invasion fitness.
We conclude that gnotobiotic and "humanized" mice represent excellent novel *C. jejuni*-infection and -inflammation models and provide deep insights into the immunological and molecular interplays between *C. jejuni*, microbiota and innate immunity in human campylobacteriosis.

Magic compounds: Ingredients of red wine, curry wurst and cholesterol-lowering drugs ameliorate acute small intestinal inflammation in mice following oral *Toxoplasma gondii* infection

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Keywords: *Toxoplasma gondii*, ileitis, anti-inflammatory properties, polyphenols, statins

The health beneficial effects of Resveratrol and Curcumin, compounds found in the red wine grape and curry spices, respectively, as well as of the cholesterol-lowering drug Simvastatin have been demonstrated in various experimental models of inflammation. We investigated the potential anti-inflammatory and immunomodulatory mechanisms of the above mentioned compounds in a murine model of hyper-acute Th1-type ileitis following peroral infection with *Toxoplasma gondii*. Here we show that after peroral administration of Resveratrol, Curcumin or Simvastatin, mice were protected from ileitis development and survived the acute phase of inflammation whereas all Placebo treated controls died. In particular, Resveratrol treatment resulted in longer-term survival. Resveratrol, Curcumin or Simvastatin treated animals displayed significantly increased numbers of regulatory T cells and augmented intestinal epithelial cell proliferation/ regeneration in the ileum mucosa compared to placebo control animals. In contrast, mucosal T lymphocyte and neutrophilic granulocyte numbers in treated mice were reduced. In addition, levels of the anti-inflammatory cytokine IL-10 in ileum, mesenteric lymph nodes and spleen were increased whereas pro-inflammatory cytokine expression (IL-23p19, IFN-γ, TNF-α, IL-6, MCP-1) was found to be significantly lower in the ileum of treated animals as compared to Placebo controls. Furthermore, treated animals displayed not only fewer pro-inflammatory enterobacteria and enterococci but also higher anti-inflammatory lactobacilli and bifidobacteria loads. Most importantly, treatment with all three compounds preserved intestinal barrier functions as indicated by

reduced bacterial translocation rates into spleen, liver, kidney and blood.
Oral treatment with Resveratrol, Curcumin or Simvastatin ameliorates acute small intestinal inflammation following oral *T. gondii* infection by down-regulating Th1-type immune responses and prevents bacterial translocation by maintaining gut barrier function. These findings provide novel and potential prophylaxis and treatment options of patients with inflammatory bowel diseases.

Cellular production of pro-inflammatory cytokines (IL-17, IL-19) and chemokines (CCL24/MCP-4) in alveolar echinococcosis patients at distinct states of infection

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Keywords: *Echinococcus multilocularis*, cellular immune response, cytokine, chemokine

With *Echinococcus multilocularis* (Em) infection the progression of disease (alveolar echinococcosis; AE) mostly occurs gradually and over extended periods of time. The classification of a regressive or progressive infection is limited, mainly depending on imaging techniques. This study evaluated the cytokine and chemokine levels in AE patients, and their parasite-specific cellular production which may associate with distinct states of infection. First, plasma concentrations of pro-inflammatory cytokines IL-17 and IL-19 and also chemokines CCL24/Eotaxin2 and MCP4 were investigated. Then, the capacity of PBMC from AE patients and infection free controls was studied in order to better distinguish between progressive, stable and cured *E.multilocularis* infection.
Plasma levels of pro-inflammatory Th2-type CCL24/Eotaxin2, mixed type MCP4/CCL13 and Th17-type IL-17B were highly elevated in AE patients as compared to controls, providing evidence that these may serve as markers for AE disease staging. In contrast, highest IL-17F were measured in controls. Following activation of PBMC with Em-antigen, peripheral blood cells (PBMC) of AE patients produced high amounts of CCL24 and MCP4, supporting the differences between patients and controls as observed in plasma. The capacity of PBMC to release these immune mediators in response to *E.multilocularis* antigens showed that concentrations of soluble IL-17R were in AE patients highly above those measured in controls. While IL-17A remained below the detection limit, IL-17B and IL-17F were produced in similar quantities in AE patients and controls. In summary, the evaluation of circulating and antigen-induced cellular production of immune mediators may provide help to better distinguish between different stages of disease in AE patients.

Session Methods and diagnostics

6 October, 2011
14.00 – 15.30

Room Lankwitz
Chair: Dag Harmsen

Gaussia princeps luciferase (Gluc) can be used to monitor type I protein secretion in *Salmonella enterica* serovar Typhimurium

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Keywords: Salmonella, pathogenicity island, T1SS, luciferase

Gaussia princeps luciferase (Gluc) is a highly active reporter exhibiting approximately 1000-fold higher luminescence compared to *Renilla* luciferase. Data about the applicability of Gluc in bacteria is very limited. Given the small size, high activity and co-factor independence of Gluc, it might be especially suited to monitor secretion of bacterial proteins. *Salmonella* Pathogenicity Island 4 (SPI-4) encodes for SiiCDF which forms a type I secretion system (T1SS) and the secreted adhesin SiiE. SiiE mediates intimate contact of *Salmonella* to polarized epithelial cells, thus facilitating the subsequent invasion process.
A codon-optimized Gluc gene was efficiently expressed in *Salmonella enterica* sv. Typhimurium (*S.* Typhimurium). Fusion proteins of Gluc and different C-terminal portions of the SPI-4-encoded adhesin SiiE were generated. After transfer in *S.* Typhimurium WT and *siiF*, luminescence was detected in bacterial lysates, supernatants and whole cultures. We detected luciferase activity of Gluc-SiiE fusion proteins in supernatants as well as whole cultures depending on a functional T1SS. We could show that a C-terminal moiety of SiiE including immunoglobulin (Ig) domain 53 is essential and sufficient mediating type I-dependent secretion of Gluc. Secretion efficiency of fusion proteins increased with increasing length of the C-terminal SiiE portion.
In conclusion, Gluc can be used to monitor protein secretion via T1SS. This is the first demonstration of enzymatic detection of secreted proteins without stable periplasmic intermediates. Gluc might open a venue for the systematic identification and analysis of type I-secreted proteins and represents a valuable addition to the toolbox of modern molecular biology and microbiology.

Peptide-microarray analysis of *in-silico* predicted epitopes for serological diagnosis of *Toxoplasma gondii* in infected humans

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Keywords: *Toxoplasma gondii*, toxoplasmosis, peptide microarray, epitope prediction, bioinformatics

Toxoplasma gondii infections in humans have a worldwide distribution. The identification of specific epitopes on *T. gondii* antigens for the detection of antibodies against the parasite is essential for improving diagnosis. In this study we examined, whether the software ABCpred is appropriate for the prediction of linear epitopes on *T. gondii* antigens. For this purpose we selected 20 peptides *in-silico* which mimick linear epitopes on GRA1, GRA2, GRA4 and MIC3 antigenic proteins. We also analysed 18 epitopes derived from GRA1, SAG1 and NTPase1 and 2, which have been described by other researchers. All 38 peptides were printed on peptide-microarray slides and analysed with human sera (n= 184), collected from patients with acute signs of toxoplasmosis (n= 21), latent *T. gondii* infection (n= 53), inactive ocular toxoplasmosis (OT) (n= 10) and from serologically positive forest workers (n= 100). Analysis revealed that 95% (19/20) of the *in-silico* predicted peptides were recognized by positive human sera, whereas all published epitopes were detected with lower diagnostic sensitivities than the predicted peptides. Univariate logistic regression suggested a significant diagnostic capability of seven predicted and two published peptides in relation to the serological status of sera. Arrays consisting of these 9 peptides had a diagnostic sensitivity of 57%

in forest workers, 79% in latently infected patients, 86% in acutely infected patients and 100% in OT patients. Analysis of seronegative sera (n=140) revealed a specificity of 84%. Our results indicate that the use of a bioinformatical approach for epitope prediction is a powerful method for discovering linear and specific epitopes on *T. gondii* antigenic proteins.

Rapid and sensitive detection of Orthopoxviruses by an Abicap-Immunofiltration column

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Keywords: diagnostics, rapid detection, orthopoxvirus, A27, affinities

As routine virus diagnostics depends on either expensive equipment or specially equipped laboratories with trained/skilled staff, rapid point of care diagnostics is not yet possible. To fill this gap we developed a simple, rapid and highly sensitive detection method for *Orthopoxvirus* (OPV), based on the Abicap immunofiltration method.
For that we tested polyclonal antibodies against several OPV surface proteins (A27, L1, F9, D8, H3, A33, B5) for their ability to detect viral particles. Monoclonal antibodies (mAbs) against A27 were generated and screened for sandwich ELISA compatibility. Biacore measurements were used to characterise the kinetic properties of the A27 mAbs. Finally, the sensitivity and cross-reactivity against different OPV strains has been evaluated on the Abicap columns.
We found that A27 was the best target for the generation of detection antibodies. The affinities of the A27 mAbs ranged between 1 and 3 nM and antibodies with fast on-rates were superior for detection purposes. Rapid detection with Abicap columns was possible for all tested OPV within 45 minutes. The limit of detection ranged from 5e3 to 1e6 PFU/ml, recombinant A27 was detectable down to 16 pg/ml. Detection from clinical samples (homogenized and/ or swabbed crusts) was possible.
The developed Abicap system is highly portable, easy to use, fast and highly sensitive for the detection of OPV. If good performing antibodies in a sandwich ELISA format are available and rapid point of care diagnostics is needed, the detection system can easily be established for other zoonotic pathogens.

Novel serological and molecular diagnostic tools for Rift Valley Fever Virus detection and surveillance

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Keywords: Rift Valley Fever Virus, recombinant proteins, monoclonal antibodies, quantitative real-time RT-PCR, ELISA

Rift Valley-Fever Virus (RVFV) is an arthropod-borne virus belonging to the *Bunyaviridae* family, genus Phlebovirus and is distributed throughout Africa and the Arabian Peninsula. The virus infects a wide range of vertebrate hosts like sheep, goats and cattle and causes significant morbidity and mortality in humans and livestock. RVFV has a serious potential to spread from Africa to Europe since there is a variety of different indigenous mosquito species in Europe that are believed to represent competent transmission vectors.

Several real-time RT-PCR (qRT-PCR) assays are available for the sensitive detection and quantification of the RVF genome. Here we describe a novel qRT-PCR protocol targeting the 3' end of the RVF genome in combination with using a novel external calibrator probe (synthetic RNA control). The performance of this new qRT-PCR is compared to those of two previously published protocols.

ELISA tests published to date are measuring the immune response to the Nucleocapsidprotein (NP). To determine the immunogenicity of other relevant RVF antigens we developed several in-house ELISAs which are based on bacterially expressed and biochemically characterised NP, Gn and Gc proteins. For this purpose polyclonal antisera against these proteins were raised in rabbits and by immunizing mice a panel of 40 monoclonal antibodies against NP and Gn epitopes were established. The performance of these ELISAs was assessed using a set of sera and corresponding negative controls from RVF infected sheep, goats, cattle and camels.

A short conserved linear epitope of influenza hemagglutinin H5 identified by recombinant antibody generation

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Keywords: antibody phage display, H5N1, chicken immune library, chicken antibodies

Influenza A/H5N1 viruses caused pandemic outbreaks in the last year. By mutation this original avian virus has aquired the ability to cross species barriers and infect humans. To date, for antibody based diagnostics of influenza anti-nucleoprotein (NP) antibodies are used. Here we show that using phage-display technology an antibody-based pathotype specific detection system can be developed.

In this work an scFv phage-library was generated using vaccinated chickens in cooperation with the Federal Research Institute for Animal Health (FLI), Germany. Eleven unique anti-H5 scFvs were selected, recloned into the scFv-Fc fusion protein format and expressed in eucaryotic cells. All of them showed binding to H5 panning target in immunoblot assays.

The vast majority of selected binders detected a large variety of H5 viruses in immunoperoxydase monolayer assays (IPMA) and showed no crossreactivity with other influenza viruses of closely related HA-subtypes.

One antibody showed very broad binding specificity for H5, not shown before, ranging from LPAIV North American strains to HPAIV viruses clade 2.2.1.

Proof of principle for a rapid H5N1 capture ELISA using infective virus preparations for diagnostic purposes was shown.

Summary:

An scFv phage-library was generated using vaccinated chickens. H5-subtype-specificity without crossreactivity with closely related HA-subtypes was shown for the majority of the fourteen selected binders. Rapid H5N1 capture ELISA using infective virus preparations for diagnostic purposes was shown.

Evaluation of antiviral activity of novel antiseptic peptides against influenza viruses

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Keywords: novel antiseptic peptides, influenza A virus infection, antiviral activity

Influenza A viruses are a continuous threat to humans as recently illustrated by the 2009 H1N1 pandemic. With increasing resistance of circulating influenza virus strains to currently available drugs, the development of novel antivirals is urgently needed. Here we have evaluated the potential of novel antiseptic peptides for their antiviral activity against influenza viruses. We found that these antiseptic peptide variants inhibit human H3N2 or mouse-adapted H7N7 virus replication by 1-4 logs in a dose-dependent manner. Further analysis revealed that some of the antiseptic peptides possess strong and specific binding affinities to sialic acids. These findings suggest that specific blockage of the cellular receptor by the peptides might reflect one possible mechanism by which virus replication is inhibited. We then assessed the inhibitory effect of these antiseptic peptides in mice. All animals, which have been simultaneously inoculated with a mouse-adapted H7N7 virus and the given peptide, survived with significantly reduced virus lung titres an otherwise 100 % lethal infection. Taken together, we could show that antiseptic peptides are promising novel candidates to target influenza virus replication *in vitro* and *in vivo*.

Session Pathogen-cell interaction I

6 October, 2011
14.00 - 15.30

Room Steglitz
Chair: Frank T. Hufert

Increased circulating endothelial progenitor cells (cEPC) in patients infected with Puumala hantavirus

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Keywords: cEPC, hantavirus, capillary leakage, endothelium

Infectious diseases often cause endothelial damage that results in increased capillary permeability or even in organ failure. A great number of zoonotic infections affects the endothelium. Therefore, the degree of damage and the mechanisms of repair play a key role in pathogenesis. cEPCs are responsible for maintenance and repair of the endothelial barrier. Several studies demonstrate that EPCs are crucial in the outcome of vascular diseases or sepsis and the use of EPCs as cell therapy is currently under investigation. However, the role of EPCs in the pathogenesis of viral infections is not yet known. Therefore, we examined the role of EPCs in hantavirus infection as an example for infectious diseases associated with endothelial damage.

We analyzed the number of EPCs in blood samples of hantavirus-infected patients with acute renal failure by flow cytometry. The number of cEPCs was significantly higher than in healthy controls (0,14%±0,063 vs. 0,04%±0,024). The analysis of EPC levels together with clinical parameters revealed that the increase of endothelial progenitor cells correlates with the clinical outcome. Courses of laboratory parameters, e.g. levels of creatinine, LDH and platelets are associated with the mobilization of EPC. After the acute stage of infection, EPC levels decreased to normal baseline values.

Further studies will analyze the levels of cytokines responsible for EPC mobilization (VEGF, EPO, GM-CSF). Together, these results will provide important insights in the pathogenesis of hantaviral infection and serve as basis for a potential therapeutic strategy using mobilization of EPCs in the treatment of infectious diseases with endothelial damage.

Puumala virus NSsORF supports replication in an Arvicolinae-derived reservoir host cell line

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Keywords: host cell restriction, IFN antagonist, antiviral

Hantaviruses persist without obvious pathogenic symptoms in their reservoir hosts. Spill over to humans however can cause life threatening diseases depending on the hantavirus species involved. Several studies suggest that modulation of the innate interferon (IFN) system by viral antagonists determines the virulence in humans. The role of this interaction for establishment of an infection in natural reservoir host cells is elusive.
To address this question a cell line derived from common vole (Microtus arvalis) was produced. The cell line designated FMF-R replicates steadily with a fibroblast-like morphology. Infection experiments with FMF-R cells were performed with the Arvicolinae-associated Prospect Hill, Tula, and Puumala (PUUV) hantaviruses. Furthermore, a PUUV variant was used, which lacks a functional NSs-ORF (Rang et al. 2006). All virus species and the variant replicated with a similar efficiency in Vero cells. In FMF-R cells increasing levels of PHV, TULV, and PUUV RNA were found after infection. Interestingly, lower levels of virus RNA were produced in cells infected with the NSs-defective PUUV variant, compared to the PUUV wild type. Differential replication of the wild type compared to the variant was confirmed on the level of the nucleocapsid protein and by titration of the infectious virions produced.
The data demonstrate that FMF-R cells are permissive for infection with Arvicolinae-associated hantaviruses. The results indicate that the NSsORF plays an important role for PUUV replication in reservoir host cells. This experimental system can be exploited to uncover specific interactions between hantaviruses and reservoir host cells.

The nonstructural protein 3 papain-like protease of a Bat-Coronavirus inhibits interferon induction and promotes viral replication in human cells

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Keywords: SARS-Coronavirus, SARS-related Bat-Coronavirus, Papain-like protease, interferon antagonist

Bats are known to be a likely reservoir for severe acute respiratory syndrome-Coronavirus (SARS-CoV). We recently identified a European SARS-related Bat-CoV (Bt-CoV) from *Rhinolophus blasii* (Drexler et al. JVI, 2010). SARS-CoV encodes several proteins that inhibit the interferon response of infected cells. The nonstructural protein 3 papain-like protease (N3plp) targets the interferon regulatory factor 3 (IRF3) by an unknown mechanism causing an interruption of the interferon-beta (IFN-b) signaling cascade.

To investigate if the homologous N3plp from a Bat-CoV (bN3plp) is also a functional IFN antagonist in human cells N3plp and bN3plp were compared with regard to their ability to antagonize the IFN-b induction in eukaryotic cell lines.

Both proteases were overexpressed in MA104 and HEK293T cells. By an interferon promoter luciferase assay their inhibitory functions were compared and showed similar inhibition of IFN-b promoter activation. The inhibition of interferon induction upon Poly I:C transfection was even stronger in case of bN3plp.

In order to investigate if this was reflecting the potential of the bat virus to replicate in human cells the bN3plp was inserted into a SARS-CoV wild-type replicon. Strikingly, the chimeric replicon carrying the bN3plp showed higher replication rates.

These results implicate that the IFN-b antagonist of a Bt-CoV does not need further adaptations to function in a human host. Further investigations examining the interplay of the antagonists in the full-virus context are needed.

Comparative analysis of the Ebola virus glycoprotein interactions in potential reservoir bat cells and human cells

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Keywords: Ebola virus, glycoprotein, reservoir, African fruit bat

Infection with Ebola viruses (EBOV) induces a severe and often fatal hemorrhagic fever in humans. Viral sequences have been detected in several African bat species which are believed to be the natural reservoir of the virus. In contrast to humans and macaques, EBOV infection of bats does not induce disease. The EBOV glycoprotein (GP) mediates viral entry into host cells and has been suggested to be a pathogenesis determinant. Here, we compared the interaction of the GPs of different EBOV species with human cell lines and cell lines from potential reservoir bat species. Our analysis focussed on i) GP cleavage by subtilisin-like proteases, ii) the intracellular localization of GP, iii) the ability of GP to induce cell rounding and iv) to counteract the antiviral factor tetherin. Finally, we examined GP-mediated entry and viral replication. We found that the GPs of the EBOV species Zaire and Sudan (ZEBOV, SEBOV) were more efficiently cleaved in human and in bat cells than the GPs of the Côte d'Ivoire and Reston (CIEBOV, REBOV) subspecies. Counteraction against human tetherin by all GPs tested was less efficient in bat cells, suggesting that EBOV-GP might need a cofactor which is not expressed in reservoir hosts. Finally, ZEBOV was able to replicate in bat cells and ZEBOV-GP was able to mediate entry into reservoir and non-reservoir cells, indicating that receptor specificity of GP might not limit EBOV tropism for bat species. Collectively, our results suggest that GP interactions with human and bat cells might not be fundamentally different.

The open reading frame 8 of SARS-Coronavirus displays host adaptation and has an influence on virus replication in primate and bat cell culture

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Keywords: SARS-CoV, SARS-related bat-CoV, ORF8

Bats have been identified as a likely reservoir of Coronaviruses, including severe acute respiratory syndrome Coronavirus (SARS-CoV). SARS-CoV encodes several accessory proteins with partially known functions in immune evasion and still unknown features.
Genomic differences of civet and human early isolate-derived SARS-CoV, human pandemic SARS-CoV, and European SARS-related bat-CoVs from *Rhinolophus* bats were identified within open reading frame 8 (ORF8). In these virus variants ORF8 is present as a single full-length reading frame, a deletion variant resulting in two different ORFs (ORF8a and 8b), or is entirely missing, respectively. In order to investigate the influence of the ORF8 adaptation in reservoir-borne versus early and late human strains we constructed recombinant SARS-CoVs (rSCVs) carrying all ORF8 variants by a reverse genetic system. Furthermore a *Rhinolophus* bat embryonic lung cell line carrying the human SARS-CoV receptor angiotensin converting enzyme 2 (hACE2) was established. Virus replication patterns of the three different rSCV-ORF8 variants were compared in bat and primate cell cultures.
On both cell cultures rSCV carrying the full-length ORF8 grew to highest titers (6 to 25-fold higher than pandemic SARS-CoV) when infected with low multiplicities of infections. The ORF8 deletion variant grew worst. The full-length ORF8 variant was least sensitive to interferon pre-treatment suggesting an increased ability to overcome the host defence mechanisms.
Interestingly, full ORF8 was missing in all sequenced strains of the European SARS-related bat CoVs, suggesting reduced virulence as opposed to Chinese strains. Future studies will investigate the mechanisms of ORF8 interactions.

Comparison of the intracellular distribution of lyssavirus matrix proteins

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Keywords: lyssavirus, rabies virus, matrix protein

Lyssaviruses are neurotropic rhabdoviruses with bats as the main reservoir. Of the lyssavirus species, only Rabies Virus (RABV) uses non-bat hosts as reservoirs, suggesting that lyssavirus species differ in virus-host interactions required for efficient host-dependent replication in vivo. In order to identify determinants of host specific replication, and referring to the recent finding that matrix (M) proteins of RABV and bat-adapted European bat lyssavirus type 1 (EBLV-1) may differ in the targeting of cellular membranes, we compared the intracellular distribution of RABV and EBLV-1 M proteins by confocal laser scan analysis. Both M proteins were detected at the plasma membrane and at intracellular structures. Surprisingly, in contrast to RABV M, EBLV-1 M colocalized with Golgi-markers, indicating that lyssavirus M proteins are able to accumulate at the Golgi apparatus and that the tested M proteins exhibit distinct phenotypes in that aspect. Moreover, beside nuclear localization of both M proteins in virus infected cells, both M proteins colocalized with nucleoprotein (N) in cytoplasmic inclusions, which are assumed to represent sites of virus RNA synthesis. In this comparative imaging approach we identified similar and distinct features of M proteins of bat and non-bat adapted lyssaviruses. This contributes to a more general understanding of lyssavirus replication. Moreover, the characterization of diverse features of lyssavirus proteins regarding their interactions with cellular components may also allow the identification of restrictive virus-host interactions and their contributions to host barriers and virus tropism.

Session Pathogen-cell interaction II

7 October, 2011
09.00 – 10.00

Room Lankwitz
Chair: Martin Beer

A single point mutation (Y89F) within the non-structural protein 1 of influenza A viruses dramatically limits lung epithelial cell tropism and virulence in mice.

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Keywords: influenza A virus, NS1 protein, SH binding motif, virulence, immune response

The non-structural protein 1 (A/NS1) of influenza A viruses (IAV) harbors several *src homology domain* (SH)-binding motifs (bm) (one SH2bm and two SH3bm), which mediate interaction with cellular proteins. In contrast to the sequence variability of the second SH3bm, the tyrosine 89 within the SH2bm is highly conserved among different IAV strains. This prompted us to evaluate the necessity of this SH2bm for IAV virulence. In an *in vivo* mouse model, we observed a dramatically reduced body weight-loss and reduced mortality upon infection with the A/NS1 Y89F mutant in comparison to wild-type virus. Infectious titers in the lung and bronchoalveolar-lavage fluid (BALF) were also reduced in comparison to wild-type virus. Concomitantly, we observed decreased cytokine, chemokine and immune cell levels in the lung and BALF as well as less severe pathological changes, reflecting reduced levels of virus-titers. Interestingly the replication of the A/NS1 mutant in mouse lung was overall reduced and strongly restricted to alveoli and if any marginally to bronchioli. In contrast, wild-type virus infection led to virus antigen positive areas in tracheal, bronchus, bronchiole and alveolar epithelium. Finally, wild-type virus infection resulted in a dramatic destruction of the bronchiole epithelium in clear contrast to infection with the A/NS1 mutant. Here, a slightly hypertrophic but entirely intact bronchiole epithelium was observed.
Taken together, we could show that disruption of the highly conserved SH2bm within the A/NS1 results in decreased virus distribution in the mouse lung and dramatically reduces virulence illustrating the necessity of the SH2bm for IAV induced pathogenicity.

Biphasic action of p38 MAPK signaling in the influenza A virus induced primary and secondary host gene response

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Keywords: MAPK p38, influenza A virus, HPAIV, cytokine storm

Highly pathogenic avian influenza viruses (HPAIV) induce severe inflammation in poultry and men. There is still an ongoing threat that these viruses may acquire the capability to freely spread as novel pandemic virus strains that may cause major morbidity and mortality. One characteristic of HPAIV infections is the induction of a cytokine burst that strongly contributes to viral pathogenicity. It has been suggested, that this cytokine overexpression is an intrinsic feature of infected cells and involves hyperinduction of p38 mitogen activated protein kinase (MAPK). Here we investigate the role of p38 signaling in the antiviral response against HPAIV in endothelial cells, a primary source for cytokines during systemic infections.

Global gene expression profiling of HPAIV infected endothelial and epithelial cells in the presence of the p38-specific inhibitor SB202190 revealed, that inhibition of p38 leads to reduced expression of interferons (IFN) and other cytokines after A/Thailand/1(KAN-1)/2004 (H5N1) and A/FPV/Bratislava/79 (H7N7) infection in both cell types. Furthermore, the expression of interferon stimulated genes (ISGs) after treatment with IFN or conditioned media from HPAIV infected cells was decreased when the target cells were preincubated with SB202190 or dominant negative MKK6 was expressed. Finally, promoter analysis confirmed a direct impact of p38 MAPK on the IFN-enhanceosome and ISG-promoter activity.

Adaptive mutations in NEP can compensate defects in RNA replication of avian Influenza viruses

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Keywords: KAN-1, NEP, pathogenicity

Transmission of Avian influenza viruses to mammals is dependent on the acquisition of adaptive mutations. Most frequently the glutamate at position 627 in the PB2 protein is mutated to a lysine (PB2-E627K) in human isolates of highly pathogenic H5N1 viruses. Interestingly, the human-derived H5N1 isolate A/Thailand/KAN-1/2004 (KAN-1) has retained the avian like PB2-E627, despite demonstrating high pathogenicity.
In this study, we used influenza database information to create a direct avian precursor (AvianPr) of the KAN-1 virus that comprises low pathogenicity in mice. Infection of BALB/c mice with reassortant viruses between KAN-1 and AvianPr revealed that the NS segment of KAN-1 increased the pathogenicity of the AvianPr in a synergistic manner with the viral polymerase of KAN-1. While we found no evidence for an involvement of NS1, NEP of KAN-1 carrying an adaptive mutation at position M16I increased the polymerase activity of the AvianPr in human cells. A complementation assay using transcription-deficient PB2 subunits revealed a defect in replication, but not transcription of the AvianPr polymerase. This replication deficit could be compensated with NEP proteins containing different adaptive mutations, derived from human H5N1 and H1N1 isolates that have retained the avian like PB2-E627. Our results therefore suggest, that NEP is a new pathogenicity factor and that mutation of NEP is an alternative strategy of influenza viruses that have not acquired the PB2-E627K mutation to adapt to the human host.

Targeting of the TRIM25-RIG-I axis by the Influenza B NS1 protein

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Keywords: influenza, NS1, RIG-I, interferon, TRIM25

The mounting of type I interferon expression is among the first and most important cellular responses to a viral infection. For influenza viruses, the RNA helicase RIG-I is the key regulator of this induction. It initially senses viral RNA and is then further activated by ubiquitination or ubiquitin chain binding and oligomerization. Previously, we and others demonstrated that the non-structural NS1 protein of influenza A viruses (A/NS1) counteracts the RIG-I-dependent interferon (IFN) induction.
The influenza B virus NS1 proteins share less than 25% amino acid identity with A/NS1. Both proteins have a set of common and diverse functions. Thus, while their N-terminal domain bind dsRNA and PKR, truncations in the C-terminus interfere with the IFN-antagonizing properties.
Here, we analyse the mechanism of B/NS1 interference with RIG-I activation. We find interactions of B/NS1 with both RIG-I and the RIG-I-activating E3 ligase TRIM25 in coimmunopreciptation and bimolecular fluorescence complementation (BiFC) assays. Furthermore, B/NS1 inhibits the TRIM25-mediated ubiquitination of RIG-I at lysine 172, as determined by electron spray ionisation mass spectrometry and the RIG-I-induced activation of the IFNβ promoter in luciferase assays.
Surprisingly, both the B/NS1 N-terminal and C-terminal domains independently bind TRIM25 in BiFC assays, which suggests a complex mode of interaction. This finding is in line with earlier work indicating that both domains have autonomous roles in IFN inhibition.
In conclusion, while these data define TRIM25 as a conserved target for different influenza virus NS1 proteins, our results suggest differences in the precise mode of its inhibition.

Session Pathogen-cell interaction III

7 October, 2011
11.00 – 12.30

Room Ballsaal
Chairs: Christian Menge and Konrad Sachse

Identification of glycolipid receptors of virulence factors from zoonotic pathogens

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

Glycolipids are abundant constituents of animal and human cells where they preferentially reside in the outward-facing part of the plasma membrane bilayer. This cell surface exposed localization renders glycolipids potential receptors for pathogens and pathogen-derived virulence factors. Many zoonotic pathogens and released toxins are known to bind, e.g., via fimbrial adhesins, to the cell surface of vertebrate cells. Although receptor-mediated interaction is a prerequisite for the infection process, their cell surface receptors remain largely unknown.
In this joint project we will set up collaborations between renowned zoonosis researchers of the National Research Platform for Zoonoses and the Glycobiology Group of the Institute for Hygiene of Münster to search for glycolipid receptors of zoonotic pathogens and their virulence factors. We will focus in the beginning of the pilot project to identify putative glycolipid receptors of subtilase cytotoxin (SubAB) of enterohemorrhagic *E. coli* (EHEC), adhesin I of extraintestinal pathogenic *E. coli* (EXPEC), avian influenza hemagglutinins H7 and H9 as well as porcine H1N1 and avian H5N1 influenza A virus strains. The project is scheduled to start in January 2012. At half time in July 2012, we will offer the colleagues of the National Research Platform for Zoonoses to provide us with any type of virulence factors for receptor investigations.
This interdisciplinary pilot project pursues a cross-linking of human and veterinary medicine. It is aimed to enhance our knowledge about zoonotic infections and could provide the basis for future development of novel anti-adhesion therapeutics, a strategy that is just at its infancy.

Synergistic adaptive mutations in the HA and PA lead to increased virulence of pandemic 2009 H1N1 influenza A virus in mice.

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Keywords: pandemic H1N1variant (H1N1v), mice-adaptation, pathogenicity determinants, polymerase activity

Influenza impressively reflects the paradigm of a viral disease in which continued evolution of the virus is of paramount importance for annual epidemics and occasional pandemics in humans. The pandemic outbreak of the H1N1variant (H1N1v) virus in 2009 caused relatively mild symptoms in the majority of patients. However, the high mutation rate of influenza viruses may facilitate to the emergence of future H1N1v strains that exhibit higher pathogenicity and cause more severe outbreaks of respiratory disease. Because of the continuous threat of novel influenza outbreaks it appears important to gather further knowledge about viral pathogenicity determinants. Here, we explored the adaptive potential of the H1N1v isolate A/Hamburg/04/09 (HH/04) by sequential passaging in mice lungs. Three passages in mice lungs were sufficient to dramatically enhance pathogenicity of HH/04, as evidenced by increased mortality in mice. Sequence analysis identified four nonsynonymous mutations in the third passage virus. Using reverse genetics, three synergistically acting mutations were defined as pathogenicity determinants, comprising two mutations in the hemagglutinin (D222G and K163E), whereby the HA(D222G) mutation was shown to determine receptor binding specificity, and the PA(F35L) mutation increasing polymerase activity. In conclusion, synergistically action of all three mutations results in a mice lethal pandemic H1N1v virus. Our results highlight the potential of H1N1v to rapidly adapt to a new mammalian host within a few passages, clearly indicating the potential for the emergence of highly pathogenic H1N1v variants.

Analyze the adaptation of influenza viruses by using well-differentiated porcine airway epithelial cells

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

Swine are important hosts for influenza A viruses playing a crucial role in the epidemiology and interspecies transmission of these viruses. Respiratory epithelial cells are the primary target cells for influenza viruses. To analyze the infection of porcine airway epithelial cells by influenza viruses, we established precision-cut lung slices as a culture system for differentiated respiratory epithelial cells. We prepared precision-cut lung slices from the lung of three months old animals. Epithelial cells of PCLS are viable for more than ten days as indicated by (i) the ciliary activity, (ii) reversible bronchoconstriction after induction by methacholine, and (iii) a live/dead viability/cytotoxicity assay. Anti-nucleoprotein antibodies were used for detection of infection by avian influenza viruses (subtypes H9N2 and H7N7). The ciliated epithelial cells were found to express both α2,3-linked sialic acid and α2,6-linked sialic acid. The growth of two avian influenza viruses (subtypes H9N2 and H7N7) was delayed by about 24 h. The two avian viruses differed both in the spectrum of susceptible cells and in the efficiency of replication. To address the adaptation process of the H7N7 and H9N2 viruses in porcine PCLS, three passages in swine PCLS were performed. The adaptation process was evident both in a shorter replication cycle and in a higher yield of infectious virus released in the supernatant of infected PCLS.

Mouse model of respiratory orthopoxvirus infection to study virulence factors essential in the development of fatal disease

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Keywords: poxvirus, pathogenesis, host-interaction, immune evasion

The emergence of zoonotic orthopoxvirus infections such as cowpox or monkeypox and the threat of a possible intentional release of pathogenic orthopoxviruses have raised new interest in the understanding of orthopoxvirus infections and the resulting diseases. Ectromelia virus (ECTV), the causative agent of mousepox, offers an excellent model system to study an orthopoxvirus infection in its natural host.
Here, we investigated low-dose respiratory infection of C57BL/6 mice with the ECTV strain Moscow. Following an incubation period of about seven days intranasal inoculation resulted in mousepox with severe conjunctivitis, respiratory symptoms, sustained weight loss, reduced motility, and 100% mortality. At day six post infection we detected high virus loads in the lungs, the draining lymphnodes, liver and spleen. Histopathologic examination revealed necrotic lesions in the epithelia of lung bronchioles and focal hepatic lesions. Moreover, upon respiratory infection of mice with an N1L deletion mutant (ECTVΔN1L) we observed a striking deficiency of ECTVΔN1L to spread from the site of primary infection to internal organs such as spleen and liver. The profound attenuation of ECTVΔN1L was reversed only by removing both CD4+ and CD8+ T cells, indicating that the presence of either cell subset was still sufficient to control the infection of the mutant virus. Thus, the N1L orthologue is an important orthopoxvirus virulence factor essential for establishment of a fatal systemic disease. The intranasal ECTV infection of C57BL/6 mice may be considered as an optimal surrogate model for severe systemic orthopoxvirus infections such as human smallpox.

Type III-secreted protein IncA of *Chlamydia psittaci* interacts with host cell protein G3BP1 to reduce c-Myc protein concentration during the chlamydial infection cycle

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Keywords: chlamydia, typeIII-secretion, Inc proteins, c-Myc, G3BP1

Chlamydia (C.) psittaci, the causative agent of psittacosis in birds and humans, is the most important zoonotic pathogen of the family *Chlamydiaceae.* These obligate intracellular bacteria are distinguished by a unique biphasic developmental cycle, which includes proliferation in a membrane-bound compartment termed inclusion. All *Chlamydiaceae spp.* possess a coding capacity for core components of a Type III secretion apparatus, which mediates specific delivery of anti-host effector proteins either into the chlamydial inclusion membrane or into the cytoplasm of target eukaryotic cells.

Here we describe the interaction between Type III-secreted protein IncA of *C. psittaci* and host protein G3BP1 in a yeast two-hybrid system. In GST-pull down and co-immunoprecipitation experiments both *in vitro* and *in vivo* interaction between full-length IncA and G3BP1 were shown. Using fluorescence microscopy, the localization of G3BP1 near the inclusion membrane of *C. psittaci*-infected Hep-2 cells was demonstrated. Notably, infection of Hep-2 cells with *C. psittaci* and overexpression of IncA in HEK293 cells led to a decrease in c-Myc protein concentration. This effect could be ascribed to the interaction between IncA and G3BP1 since overexpression of an IncA mutant construct disabled to interact with G3BP1 failed to reduce c-Myc concentration.

We hypothesize that lowering the host cell c-Myc protein concentration may be part of a strategy employed by *C. psittaci* to avoid apoptosis and scale down host cell proliferation.

The Function of the H_{CN}-domain of clostridial neurotoxins

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Keywords: Tetanus neurotoxin, Botulinum neurotoxin, H_{CN}-domain, binding, biological activity

The family of clostridial neurotoxins (CNTs) consists of tetanus neurotoxin (TeNT) and the seven botulinum neurotoxin serotypes (BoNT/A-G). They are composed of four functionally independent domains that perform individual tasks in the intoxication process. The functions of the 50 kDa catalytically active light chain, the 50 kDa translocation domain H_N and the C-terminal 25 kDa H_{CC}-domain binding to two neuronal receptors are resolved but the role of the 25 kDa H_{CN}-domain remains unclear.
Deletion or substitution of the HCN-domain may provide insight into its function. At first, mutants of BoNT/A, BoNT/B and TeNT were generated either lacking their H_{CN}-domains or containing H_{CN}-domains from one of the other two serotypes. Their residual biological activity was tested using the mouse phrenic nerve hemidiaphragm (MPN) assay. Replacement of the H_{CN}-domain in TeNT by H_{CN}A and H_{CN}B or its deletion similarly reduced the biologic activity by about 95 %, whereas BoNT/A and B deletion mutants displayed less than 0.3 % activity. Swapping H_{CN}-domains between BoNT/A and B does not impair their activity, but substitution with H_{CN}T clearly decreased the biological activity. Additionally, the ganglioside and protein receptor interaction of the mutants was verified.
In conclusion, the exchange of H_{CN}-domains between different BoNT serotypes is feasible without impairing the biological activity whereas the structurally similar the H_{CN}-domain of TeNT cannot equally substitute those of BoNT and vice versa, leaving the possibility that H_{CN}T plays a different role in the intoxication mechanism of TeNT.

Session Epidemiology, surveillance and risk assessment I

7 October, 2011
09.00 – 10.00

Room Ballsaal
Chairs: Dirk Schlüter and Lothar Wieler

Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from poultry meat

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Keywords: retail meat, foodborne pathogen, antimicrobial resistance, virulence, microarray analysis

Background: During recent years, MRSA have been detected not only in animals but also in food. Since there is little information available about MRSA from food in Germany, the aim of this study was to characterize MRSA from food of poultry origin.

Methods: In a survey, 32 of 85 samples of fresh meat/meat products of poultry origin were MRSA-positive. For these isolates *spa*, SCC*mec* and *dru* typing and two CC398-specific PCR assays/MLST were performed. MICs were determined by broth microdilution. Resistance and virulence genes were detected by a *S. aureus*-specific DNA microarray and by PCR.

Results: Twenty-eight isolates belonged to CC398, two to ST9 and single isolates to ST5 and CC5/ST1791. The CC398 isolates carried SCC*mec* elements of types IV and V, showed five *spa* (t011/t034/t899/t2346/t6574) and ten *dru* types (dt2b/dt6j/dt6m /dt10a/dt10as/dt10at/dt10q/dt11a/dt11v/dt11ab). Both ST9 strains had SCC*mec* type IV, *spa* type t1430 and *dru* type dt10a, whereas the ST5 and the ST1791 strains had SCC*mec* type III, *spa* type t002 and *dru* type dt9v. Thirty isolates showed resistance against at least three classes of antimicrobial agents. The respective resistance genes *mecA*, *blaZ* [β-lactams], *tet*(K/L/M) [tetracyclines], *erm*(A/B/C/T), [macrolides /lincosamides/streptogramin B], *dfrS1*/*dfrK* [trimethoprim], *aacA-aphD*, *aadD* [aminoglycosides], *apmA* [aminocyclitols], *vga*(A/C) [lincosamides/pleuromutilins/streptogramin A] were detected. All isolates were negative for PVL, TSST-1 and exfoliative toxin genes. The ST9, ST5 and ST1791 isolates harboured the enterotoxin gene cluster *egc*.

Conclusions: The presence of MRSA, including enterotoxigenic strains, in 37.6% of the samples is alarming. Further studies are

needed to investigate possible health hazards and routes of transmission.

Comparative genetic analysis of canine and human *S. aureus*-isolates reveals a substantial proportion of extended host spectrum genotypes (EHSG)

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The general concept of *S. aureus* ecology is host specialization. In contrast to this paradigm, comparative molecular typing results of *S. aureus* strains (including MRSA) from human and various animal origins indicate the possibility of cross-species transmission of MRSA strains, defined as extended host spectrum genotypes (EHSG).
The aim of this study was to conduct a broad comparative molecular analysis with regard to the genetic composition of clinical canine and human *S. aureus* strains, including *mec*A-positive (MRSA) and negative (MSSA) representatives. Initially, 96 canine *S. aureus* isolates of various geographic origins underwent *spa*- typing, pulsed field gel electrophoresis (PFGE), microarray hybridisation (MH) of 170 genes and multilocus sequence typing (MLST; for selected isolates). For further comparative analysis, human strains were included which belonged to the same spa-types as commonly observed in canine isolates. Analyzing the typing results by applying a UPGMA clustering based on the Dice coefficient for PFGE and the *Pearson correlation* for MH using BioNumerics software *(Applied Maths NS)* revealed that the vast majority of *S. aureus* strains obtained from dogs showed PFGE- and MH-typing pattern which cluster with human isolates.
These data seem to reflect a close human-to-dog relationship allowing interspecies transmission of clinically relevant *S. aureus* strains by frequent body contact (e. g. petting the dog) in general. Therefore, EHSG should be considered as a potential factor especially in cases of recurrent MRSA- infection in both, human or canine household patients.

Adherent-Invasive *Escherichia coli* (AIEC) – a group of pathogens associated with Crohn's disease in humans – are frequently isolated from enteritis in cats, dogs, and swine

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Adherent-Invasive *Escherichia coli* (AIEC), which have been associated with Crohn's disease, show traits similar to human and animal extraintestinal pathogenic *E. coli* (ExPEC) regarding their phylogenetic origin and virulence gene profile. To unravel a possible role of animals as source of AIEC strains, we analyzed the AIEC phenotype of 79 ExPEC strains from birds, cats, dogs, and swine, which were selected with respect to multi locus sequence types (STs) that had previously been linked with AIEC strains from patients with Crohn's disease, including ST131, ST73 and ST10. In addition, 45 animal *E. coli* enteritis isolates of ST complexes 10 and 73, the latter one representing a prominent lineage linked with urinary tract infections and septicemia in humans, were included.

While only 1.3% of ExPEC strains showed an AIEC phenotype, as shown by the ability to adhere to and to invade intestinal epithelial cells, as well as to survive and replicate within macrophages, 57.8% of animal enteritis strains (33.3% swine; 35.3% dogs; 81.8% cats) phenotypically resembled the AIEC pathotype. Interestingly, none of the STC73 ExPEC strains showed this phenotype, whereas 72.4% of STC73 strains from enteritis were confirmed as AIEC-like strains. Due to the non-arbitrary strain selection no conclusion can be drawn considering the real prevalence of AIEC-like strains in the intestinal tract of animals, but their frequent detection provides evidence for a lack of host-specificity and a zoonotic risk. These findings underline the urgent need of future studies on the epidemiology, phylogeny and genotypic characteristics of AIEC-like strains from animal sources.

Genetically different *T. gondii*, isolated from a single feline faecal sample from Germany, have different virulences in BALB/c mice.

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Keywords: *Toxoplasma*, PCR-RFLP, genotype, isolation, virulence

Toxoplasma gondii is a protozoan parasite that infects a number of warm-blooded vertebrates, including humans. Wild and domestic felids act as definite hosts. They shed the oocyst stage which becomes infectious after sporulation. Intermediate hosts such as rodents, foxes, livestock animals, birds and humans become infected by either taking up oocysts or by consuming other infected intermediate hosts. *T. gondii* has a clonal population structure. In Europe, three clonal lineages of *T. gondii*, referred to as clonal types I, II and III, are observed. A single type I parasite is sufficient to kill a mouse whereas more than 1000 parasites of types II and III are needed to achieve the same effect in mice. Analysing feline faecal samples from Germany we predominately found *T. gondii* of clonal type II but also observed type III and recombinant genotypes. A single faecal sample contained many recombinant and genetically different *T. gondii* displaying various combinations of type II- and type III-alleles at different loci. Individual clones were identified by limited dilution and shown to possess different virulence phenotypes in mice. The majority of clones were of high virulence for mice. Recombinant *T. gondii* are the major cause of ocular toxoplasmosis in North and South America. Our study shows that new, genetically recombinant *T. gondii* with different virulences can form under natural conditions in Germany.

Session Epidemiology, surveillance and risk assessment II

7 October, 2011
11.00 – 12.30

Room Lankwitz
Chair: Christian Drosten

A high prevalence of HEV-specific antibodies observed in slaughterers may raise the question of HEV transmission during the process of slaughtering

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Keywords: zoonosis, genotype, immunoassay, seroprevalence

An increasing number of autochthonous hepatitis E virus (HEV) infections were observed in Germany. These cases are believed to be the result of a zoonotic HEV transmission from pigs, wild boars and deer. Recently, a high prevalence of HEV-specific antibodies in the German domestic pig population has been demonstrated. Thus, humans with occupational exposure to pigs may be at a higher risk for HEV infection.

In this study, sera obtained from 24 slaughterers, 14 meat inspectors, 46 pig farmers and 22 veterinarians were tested for the presence of HEV-specific antibodies. For comparison, sera obtained from 116 age and gender matched blood donors were also included. 28.3 % (30/106) of the swine-exposed humans and 15.5% (18/116) of the blood donors without contact to pigs exhibited IgG-antibodies determined as reactive against HEV. Thereby, an increased risk for HEV infection in humans professionally exposed to pigs and particularly for slaughterers (41.7%; 10/24) was demonstrated.

It is hypothesised that a direct contact to HEV-rich tissues and fluids during the process of slaughtering should be considered as another risk factor of HEV transmission. The unexpected high HEV seroprevalence observed in blood donors could lead to a re-evaluation of the risk of HEV exposure among the general population.

Rats as potential reservoir for cowpox virus infections

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Keywords: feeder rats, cowpox, serology, zoonotic infections

In the last three years the number of cowpox virus (CPXV) infections in Germany has highly increased.
CPXV is rodent borne with a broad host range. It contains the largest and most complete genome off all poxviruses, including parts with a high homology to variola virus (smallpox).
Due to several CPXV outbreaks among zoo animals and humans after contact to infected rats between 2008 and 2009, the attention regarding the transmission of CPXV through rats has thoroughly increased. Whether wild and domestic rats are a primary reservoir or just amplifying hosts for CPXV has not yet been revealed.
In the outbreaks mentioned above, domestic rats - bred on different farms throughout Europe as food for carnivores - have been identified as the source of CPXV infections.
Rats of different farms and of four different age categories (baby, small, adult, breeder) were analyzed for CPXV. Blood was tested for antibodies against CPXV by IFAT; lung and liver where tested by real-time PCR for existing CPXV-DNA.
In one farm, all animals tested showed antibodies specific against CPXV, whereas virus DNA was found in adult and breeder rats only with a prevalence of 55 % and 72 %, respectively.
Since none of the examined animals showed any pathological signs of disease, a possible asymptomatic course of a CPXV infection can be suggested. How the virus found its way into the domesticated rat population is still unknown. Nevertheless, these results indicate that rats certainly play an important role in transmitting CPXV.

In search for factors driving hantaviruses and rickettsia – studies in an climate-altitude gradient

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So far there is a lack of longitudinal studies on rodent-associated pathogens and to understand the factors that drive the oscillations of these agents. We therefore in the last three years investigated 657 rodents along an altitude gradient ranging from 300m above sea level (asl) up to 1450 m asl in the national park of the Bavarian Forest at the frontier to the Czech Republic. Sampling sites were selected from the already ongoing BIOKLIM-project in which many data on the biotopes are collected, e.g. flora, fauna, several climatic data for comprehensive statistical analyses. Local meteorological data such as temperature, dew point and humidity are recorded at each site. During section of the rodents ectoparasites and several biometric data of the animals were collected for statistical analyses. Samples are processed and screened by serological and molecular biological assays for hantaviruses and rickettsia. The prevalence rate of the two agents ranged from 2 up to 60%. In some regions, rodent trapping indices and PUUV occurrence in bank voles seem to reflect the dynamics of human case reports. For *Rickettsia felis* and *Rickettsia helvetica* we could show that rodents seem to be a widely spread reservoir. Presently, all data are analyzed in a Bionumerics database, and ArcGIS and regression analyses are performed in order to identify possible risk factors for the occurrence of hantaviruses and the prevalence of human hantavirus infections. The investigations on the abundance of hantaviruses include genetic diversity, genetic reassortment, the host range and environmental (flora, fauna) and climatic factors influencing the prevalence and incidence of human infections in selected regions. In conclusion, the results of the study will allow the identification of potential risk factors for human health as a basis for disease surveillance. This work is part of the VICCI-network supported by the Bavarian Ministry of Health.

Zoonotic behavior of the pandemic swine influenza virus in Switzerland

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Keywords: Swine influenza virus, epidemiology, pig, zoonosis

The origin of the pandemic influenza virus that emerged in Mexico in 2009 has been blamed to pigs, giving rise to the term "swine influenza".
We made use of 49 swine influenza virus (SIV) isolates, collected between 2004 and 2010 from clinically affected pigs to ask, whether or not the pandemic SIV did circulate and caused illness among the Swiss pig population. Therefore, we applied the multisegment reverse transcription PCR (M-RTPCR) by Zhou et al. (2009) on our isolates. The amplification products were sequenced. Sequences of a total of 55 segments were determined, the numbers decreasing with increasing sizes of the segments. According to these sequences, all Swiss SIV isolates were H1N1 but clearly distinct from the pandemic strain. Segment 7 (M1) was most highly conserved among all isolates, whereas segment 4 (HA) was most variable. The array of sequence identity ranged from 90 to 99% among the Swiss strains but dropped to levels between 62 to 90% as soon as the pandemic strain was included in the comparison.
Overall, our results indicated that before 2010 the circulating Swiss SIV strains were clearly distinct, not only from the pandemic SIV but also from conventional European SIV strains. However, since early 2011, we have started detecting the pandemic SIV in swabs from diseased Swiss pigs but only long after the same strain had begun to circulate among the Swiss people. Thus, the pandemic SIV seemed to have been transmitted from humans to pigs and not vice versa, at least in Switzerland.

Phylogenetic analysis of yellow fever virus isolates from West Africa

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Keywords: yellow fever virus, epidemiology, Africa, full genome, growth behaviour

Yellow fever is still a major health problem of the world. Until now there are five known genotypes of yellow fever virus (yfv) circulating in Africa, belonging to only one serotype. Due to limited access to new isolates, the knowledge about the genetic diversity and evolutionary dynamics of YFV is very low. Also the effect of certain mutations on the phenotype is not studied very well. More recent studies, based on E-protein sequencing, could show the circulation of at least six different YFV-lineages only in West-Africa. To broaden these findings, we sequenced the full genome of representatives of these different lineages to examine the phylogenetic correlation and performed growth kinetics on mosquito and human liver cells to further analyse the phenotypes. The phylogenetic analysis of the complete genome supports the clustering in the six lineages. In mosquito cells, all lineages sequenced here differed in their growth behaviour from the reference strains YFV-Asibi and -17D. Interestingly, lineage three does not replicate very well in mosquito cells. This lineage is previously known to be associated with vertical transmission in its natural vector. In human liver cells we can not observe such differences between the tested strains.
Our sequencing supports the finding that there is a wider genetic diversity of YFV existent in Africa than suggested before. Further sampling and sequencing could improve our knowledge about the evolutionary processes of yellow fever virus and thereby could serve as a tool for the understanding of outbreaks and the interplay in its zoonotic cycle.

Molecular epidemiology of rabies in the Middle East and Central Asia with special emphasis on Iraq and Afghanistan

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Keywords: rabies, zoonosis, Middle East, phylogeny

Rabies is a major public health burden in the Middle East and Central Asia with the primary reservoir being the domestic dog. Little is known on the circulating virus strains especially in countries such as Iraq and Afghanistan where political instabilities prevent systematic disease surveillance. In the frame of the mandate of the U.S.A in both countries, 34 brain samples of five different species from Iraq and Afghanistan were diagnosed by the U.S. army veterinary laboratory Europe using dRIT and confirmed with the recommended DFA. To gain insights into the molecular epidemiology of rabies in this region these isolates were amplified by PCR and partially sequenced. Further archived samples of the FLI, ECVCR, and AHVLA from Turkey, Pakistan; Oman, Emirates, Saudi Arabia and Azerbaijan were also analyzed. Sequence comparison and phylogenetic analysis revealed at least three distinct lineages in this region. Lineage "A" seems ubiquitous in the Middle East with isolates from many different countries, e.g. Iran, Iraq, Oman, Saudi Arabia, Israel and Jordan. Lineage "B" contains isolates from eastern Turkey, northern Iran, Georgia and Iraq and had not been identified as a separate phylogenetic lineage prior to this study. Viruses of the third lineage that seems to circulate in this region including Afghanistan are referred to as "arctic-like". Thus far "arctic like viruses" seem the only prevalent RABV strain in Afghanistan and Pakistan.

Session Wildlife and new or re-emerging zoonoses I

7 October, 2011
09.00 – 10.00

Room Steglitz
Chair: Sandra Eßbauer

Emerging infectious diseases: Impact of anthropogenic change in land use on prevalence of blood parasites in Neotropical bats

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Keywords: bats, habitat change, trypanosomes, Chagas' disease

In our study, funded by the BMBF under the umbrella of the Nationale Forschungsplattform für Zoonosen, we investigate effects of anthropogenic change in land use on prevalence of bat blood parasites. Changes in land use associated with the decrease of biodiversity have been linked to the emergence of zoonoses. Bats are hosts of a variety of human pathogens, including the etiologic agent of Chagas' disease, *Trypanosoma cruzi*. Currently, Chagas' disease is re-emerging in Central and South America. In Panamá, we found a drastic increase in *T. cruzi*-like trypanosomes in a bat species that occurred at high numbers in species-poor, disturbed habitats compared to undisturbed forests with high bat diversity and low pathogen load. We are expanding our approach towards community level studies and are focusing mainly on trypanosomes and their main hosts in habitats reflecting different stages of human interference: 1) undisturbed, continuous forest, 2) forested islands and 3) disturbed forest fragments close to human settlements. We take blood from bats in the field and subsequently check it quantitatively and qualitatively for haemoparasites with a microscope and a high- resolution photographic unit. Furthermore, we perform molecular diagnostics for detection and identification of strains and take trypanosomes into culture. Specifically, we want to test the *dilution effect* about the assumed protective effects of biodiversity, and we aim at identifying factors affecting the re-emergence of Chagas' disease. Ultimately, we want to gain a better understanding about the importance of biodiversity as a potential buffer for humans against spread of disease and ecosystem health.

Henipavirus RNA in bats from bushmeat markets

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Keywords: bats, bushmeat, Henipa, Eidolon, paramyxovirus

Henipaviruses (Hendravirus –HeV- and Nipahvirus -NiV-) are zoonotic Paramyxoviruses capable of causing fatal disease in humans. Flying foxes (*Pteropus* ssp.), occurring in Australia and South-east Asia, serve as reservoir host for both viruses but Henipavirus RNA and antibodies have also been described in the straw-coloured fruit bat *Eidolon helvum* in Ghana, West Africa. *E. helvum* is a large migratory fruit bat, highly abundant in sub-saharan Africa, and regularly hunted and consumed by humans. To investigate the risk of zoonotic disease emergence via the preparation and consumption of bushmeat we bought 42 animals (all specified as *E. helvum*) from hunters providing animals to a market in Brazzaville, Republic of Congo. A standardized sample set was used for RT-PCR screening for Henipavirus RNA targeting the L-gene. Different organs of several animals were found to harbour viral sequences, all related to Henipaviruses. So far, no human case of Hendra- or Nipahvirus infection has been reported from Africa but low local health care standards might contribute to undiagnosed cases. Hunters, merchants and consumers are frequently exposed to bat excretion and blood and thus at high risk for spill-over infections. Besides further studies on virus-host-ecology, a serological survey in exposed persons would help to estimate the zoonotic potential of bat paramyxoviruses that seem to be common in *E. helvum*.

Bat and canine adenoviruses: possible ancient inter-species transmission?

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Keywords: bat, adenovirus, host adaptation, inter-species transmission, canine adenoviruses

Emerging zoonotic diseases are a major threat to public health and many of them are associated with bats.
In 2008, a novel adenovirus (bat AdV-2 strain PPV1) was isolated via cell-culture from organ tissue material of a noctule bat. This virus was further characterized by 454-sequencing, genome organization and phylogenetic analyses, which resulted in the finding of a close relationship to canine adenoviruses (CAdVs). Bat AdV-2 strain PPV1 was unexpectedly found carrying genes (in the E3 and E4 regions) that where previously exclusively identified in canine adenoviruses. Although adenoviruses are generally strictly species-specific and only moderately pathogenic for their hosts, doubts have been raised for canine adenoviruses. CAdV-1 is unusually highly pathogenic for dogs and has unexpectedly been isolated from other carnivores. This high pathogenicity and the easy crossing of the species barrier might be a sign of insufficient host adaptation. Due to the close genetic relationship between bat and canine adenoviruses, a possible ancient inter-species transmission is presumed and will be discussed. To possibly prove this hypothesis, further experiments were performed. CAdV-1, bat AdV-2 and, for comparison, human AdV-4 were titrated in the xCelligence® system on different human, dog and monkey cell-lines to characterize their host specificity. The genes of the viral hexon, fibre and penton base proteins, which are important for the antigenicity and receptor binding, will be re-sequenced and compared to bat AdV-2 genome to identify possible genetic changes during cell-culture infections. Additionally, an *in vitro* neutralization assay with antibodies against CAdV-1 and bat AdV-2 was performed to study possible serologic cross-reactivity between CAdV-1 and bat AdV-2. This study might bring further insight into inter-species adenoviral transmission to dogs.

Bat rabies in Germany - discovery of a novel lyssavirus in a Natterer's bat (*Myotis nattereri*)

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Keywords: Novel bat lyssavirus, Myotis nattereri, bat rabies

More than 230 cases of bat rabies have been reported in Germany with the vast majority found in Serotine bats (*Eptesicus serotinus*) and characterized as EBLV-1. Recently, the presence of EBLV-2 in Daubenton's bats (*Myotis daubentoni*) from Germany was confirmed.
Here we report the first documented rabies case in a Natterer's bat (*Myotis nattereri*). The bat was initially found during daytime in November 2009 and was taken to a regional bat sanctuary. In February 2010 the bat began to behave aggressively, showed targeted, directed approach to any moving object, and tried vigorously to bite. Ten days after the recognition of first clinical signs the bat succumbed and was submitted for rabies diagnosis. Initial FAT results were corroborated by immune-histochemistry and RTCIT. However, the reaction pattern of the isolate performed with a panel of 10 anti-nucleocapsid mAbs in cell-culture clearly differentiated the isolated virus from EBLV-1 and 2 and other lyssaviruses based on positive reactions of mAbs W239.17, W187.11.2, MW187.6.1 and MSA6.3. Also, EBLV-1 / 2 specific RT-PCR and realtime RT-PCR failed to detect viral RNA and only a hemi-nested RT-PCR gave a positive result. Subsequently, partial and full genome sequence analysis followed by phylogenetic analysis supported the assumption that the isolated virus, designated Bokeloh bat lyssavirus (BBLV), may represent a novel member of the lyssavirus genus. Since the Natterer's bat is a widespread and abundant bat species in Europe the public health importance of this new virus needs to be elucidated.

Session Wildlife and new or re-emerging

zoonoses II

7 October, 2011
11.00 – 12.30

Room Steglitz
Chair: Gudrun Wibbelt

Discovery of polyomaviruses in non-human primates: indicators for the existence of unknown human polyomaviruses

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Background: Polyomaviruses (PyVs) have been discovered in humans, non-human primates and other vertebrates. In humans, nine PyVs are known, and four of them (JCV, BKV, MCPyV, TSV) are known to cause disease, in particular in immunocompromised patients. The aim of the present study was to further unravel the diversity of primate PyVs, and to obtain indications for additional unknown human polyomaviruses.
Methods: Polyomavirus VP1 sequences were amplified from primate samples, collected in Africa and Europe, with universal PyV-PCR using degenerate primers. PyV-positive samples were subjected to long-distance PCR to amplify the remainder of the circular PyV genome. Complete PyV genomes and partial PyV sequences were analysed using multiple sequence alignments and phylogenetic tree construction.
Chimpanzee and human sera were tested for antibodies against chimpanzee PyVs in a recombinant ELISA based on E. coli-expressed VP1 proteins.
Results: A plethora of novel PyVs (n=18) were discovered in great apes, Old World monkeys and New World monkeys. They cluster in several groups containing either only non-human primate (NHP) PyVs or both NHP-PyVs and human PyVs. Serological testing revealed that chimpanzees are sero-positive for their PyVs. Testing of human sera for sero-reactivity against these chimpanzee PyVs possibly indicating cross-reactivity with unknown human PyVs are on the way and will be presented.
Conclusions: There are more distinct groups of primate PyV than presently known. They may still have yet unknown members which are hosted by the human population.

Infection studies with West Nile virus lineage 1 and 2 in large falcons

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Keywords: West Nile virus, experimental infection, falcons, lineage 1 and 2

West Nile virus (WNV) is an important zoonotic flavivirus that is transmitted by blood-suckling mosquitoes and uses birds as primary vertebrate reservoir hosts (enzootic cycle). Some bird species like ravens, falcons and jays are highly susceptible and develop deadly encephalitis while others are only going through a subclinical infection.
Findings in the past indicated a high susceptibility of raptors for both WNV lineages (WNV lineage 1 and the Austrian/Hungarian WNV lineage 2 isolate). The aim of this experimental study was to evaluate the effect of WNV lineage 1 (NY99) and 2 (strain Austria) using three different virus doses in falcons to clarify the role of these species in WNV enzootic cycle.
For the infectious trial we used for each virus lineage six captive-bred hybrid falcons (Falco rusticolus x Falco cherrug). Always two falcons were subcutaneously inoculated with low, intermediate or high dose of WNV-NY99 or WNV-strain Austria. Blood samples, cloacal and oropharyngeal swabs were collected at 2, 4, 6, 10 days post infectionem (dpi) and at the end of the experiment after 2 respectively 3 weeks. Clinical signs were observed over time and birds were necropsied between 14 and 16 dpi (lineage 1) or 20 and 21 dpi (lineage 2). All experiments were carried out under biosafety level 3 conditions.

Following the challenge with virus from both lineages falcons developed clinical signs and typical gross pathological findings were observed. Detailed results of virus re-isolation, qRT-PCR and serology will be presented for a range of tissues and bodily fluids.

Characterization of an emerging Mongolian TBE virus strain

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Keywords: tick borne encephalitis virus, Mongolia, *Ixodes persulcatus*

Tick-borne encephalitis virus (TBEV), a member of the family *Flaviviridae*, poses an increasing health and economic problem in Mongolia. Endemic areas of TBE have been reported mainly in the provinces Selenge and Bulgan in Northern Mongolia close to the Russian border. We report the first isolation and characterization of a TBEV strain from ticks collected in Mongolia.
68 ticks (*Ixodes persulcatus*) were collected by flagging in the Bulgan district near Khylganatt in the North of Mongolia in July 2010. Extracted RNA was screened for TBEV-specific sequences by real-time RT-PCR. Two out of 68 (2.9%) tested ticks RNAs were reactive. The real-time RT-PCR positive tick supernatants were inoculated into Vero cells and the TBEV strain (MucAr M14/10) could be recovered.
After full-genome sequenzing the comparison of the 1488 nt long E-gene revealed the highest homology on the nucleotide level (96.7%) and on the amino acid level (99.5-99.7%) to three strains of a subclade of the Siberian subtype of TBE virus, the strains Zausaev, the strains IR99-2m7 and Lesopark, respectively. MucAr M14/10 was passaged in Vero B4 cells to determine the titer and the growth kinetics generating a one-step growth curve. Cytopathic effects and plaque morphologies were investigated and compared with different TBE viruses.
In conclusion we describe the first isolate of a TBEV in Mongolia that was full-genome sequenced and biological characterized. Because of the close relationship with strains along the Trans-Siberian railway we postulate that the transmission of TBE in Mongolia has taken place along this route.

The German arbovirus surveillance and mosquito monitoring program, 2009 - 2010

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Keywords: arbovirus, Germany, mosquitoes, Sindbis virus, Batai virus

The aim of the program is to provide an early warning of the presence of arboviruses in Germany, in an effort to reduce the potential for human or animal disease. The program compiles and analyses mosquito and arbovirus data collected over a number of successive years. This will provide a solid base to determine the underlying causes of the seasonal fluctuations in arbovirus activity and the relative abundance of the mosquito vector species. This information can then be used as a basis for vector control programs. During 2009 and 2010 we monitored mosquito vector populations and undertook surveillance of arbovirus activity mostly in South West Germany. Approximately 90,000 mosquitoes were captured and assayed for the presence of arboviruses. In 2009, Sindbis virus (SINV) and Batai virus (BATV) were isolated from *Culex* spp. and *Anopheles maculipennis s.l.*, respectively. The highest SINV infection rate (4.9) in the *Culex* mosquitoes was in the beginning of July. Phylogenetic analysis of the German SINV strains linked them with Swedish SINV strains, the causative agent of Ockelbo disease in humans. Analysis of partial S, M, and L segments of the German BATV strain showed that the sequences from all three segments were most closely related to BATV, indicating that the virus has not undergone reassortment. In contrast, only Usutu virus (USUV) was isolated in 2010 from *Culex* spp. and demonstrated to be related to USUV strains circulating in Austria and Italy. Further studies have to be conducted to estimate the veterinary and medical importance of SINV, BATV and USUV in the affected areas.

Interdisciplinary zoonosis research in Germany: the network "Rodent-borne pathogens"

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Keywords: zoonoses, reservoir host, rodent, hantavirus, hepatitis E virus

The network „Rodent-borne pathogens" was established as a platform for a broad interdisciplinary collaboration. The major objective of the network is to increase the collaboration between research teams working on different rodent biology aspects or on the molecular epidemiological characterization of zoonotic pathogens. A total of about 11,000 rodents and other small mammals were collected in 15 federal states by the trapping efforts of the Friedrich-Loeffler-Institut, Julius Kühn-Institut, Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, forestry institutions of several federal states and additional collaborators. The molecular epidemiological pathogen studies in small mammals were focussed on the prevalence of already known zoonotic pathogens and the search for novel rodent-associated pathogens. These studies resulted in initial data on the presence of zoonotic pathogens, i.e. hantaviruses, orthopox viruses, tick-borne encephalitis virus and *Leptospira* species in small mammals. Recently, the network was involved in molecular epidemiological studies on the hantavirus outbreak in Germany in 2010. In addition, novel rodent-associated pathogens have been identified, e.g. a novel hepatitis E virus in the Norway rat.
In the future the investigations of the network should prove potential associations between population dynamics, population genetics and pathogen prevalence in small mammals. Therefore longitudinal rodent monitoring studies were initiated in 2010 at selected sites in Baden-Wuerttemberg, North Rhine Westphalia, Thuringia and Mecklenburg-Western Pomerania. In addition, outbreak and molecular epidemiological studies in reservoir hosts and patients will be intensified by a closer collaboration of veterinary and human medicine.

Multiresistant *Escherichia coli* from brown rats: phylogenetic background and virulence potential

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Keywords: ExPEC, multiresistance, MLST

The re-emergence of urban rats as a global public health concern and their possible role in the spread of antimicrobial resistance lead to the design of a pilot study in order to determine the phylogenetic background and virulence potential of rat-derived multiresistant *Escherichia coli*. A total of 212 faecal *E. coli* isolates, obtained from 85 wild urban brown rats (*Rattus norvegicus*) were screened for phenotypic antimicrobial resistance. After exclusion of repetitive clones via RAPD and PFGE and phenotypic resistance determination 29 multiresistant isolates were further characterized via multi locus sequence typing (MLST), structure analysis of ancestral groups and for genotypic resistance as well as virulence determinants. MLST revealed a broad diversity of sequence types (n=23 STs) among the multiresistant strains including those which have previously been associated with extraintestinal pathogenic *E. coli* (ExPEC), such as ST70 (ancestral group D), ST428 (B2) and ST127, ST95 (B2). Accordingly, we observed a number of virulence associated genes typical for ExPEC, whereas virulence determinants known for intestinal pathogenic pathovars were only infrequently observed. Our findings demonstrate that multiresistant *E. coli* are commonly present among urban rats. In addition, some of these isolates display a combination of genotypic and phylogenetic determinants which confer not only a multiresistant but also a virulence- or fitness-related phenotype, the latter probably facilitating their assumed persistence in the rat intestine. Due to the close contact between rats and humans interspecies transmission events may accelerate and besides the classic rat-derived pathogens like *Leptospira* the spread of other multiresistant pathogens, such as ExPEC, should also be kept in mind.

Poster Presentations

Poster Session – Innate and adaptive immune response

I01

Comparison of the interferon response of bat cells with prototypic mammalian cell cultures

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Zoonotic viruses like severe acute respiratory syndrome (SARS)-Coronavirus, Ebola- and Marburgvirus, which are responsible for severe human diseases, are most likely hosted by bats. Since bats do not seem to show any clinical symptoms the question raises if they have possibly evolved unique immunological mechanisms to avoid viral replication. The first intracellular defence mechanism to virus infection is the interferon (IFN) response. In this study we compared the IFN response of two bat kidney cell lines (RoNi, *Rousettus aegyptiacus* and EidNi, *Eidolon helvum*) with three prototypic mammalian cell lines (MA104, primate; MEF, mouse; A549, human).
All cells were tested for a functional IFN pathway by analysing IFN induction, secretion and signalling. IFN induction was triggered by two known inductors, poly IC and Rift Valley Fever virus clone 13. IFN mRNA levels were quantified by real-time PCR. IFN secretion was measured by a VSV bioassay. IFN signalling was analyzed with the help of real-time PCRs for IFN stimulated genes (ISG) like MxA and ISG56.
Subsequently the IFN response of all cells was compared upon O´nyong nyong virus (ONNV) infection, an alphavirus, which is known to strongly induce IFN. In most cell lines ONNV replication led to pronounced IFN mRNA induction but resulted in relatively low IFN protein secretion and IFN signalling. Interestingly, RoNi cells showed minor viral replication but a higher IFN secretion and signalling level. These results suggest differences in the IFN response between bat species. Future studies will address if different cell cultures from *Rousettus aegyptiacus* show similar features.

I02

Rig-I activates the Phosphatidylinositol-3-kinase (PI3K) to promote efficient type I interferon production in response to influenza virus vRNA

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Keywords: influenza viruses, Rig-I, vRNA, PI3K signaling, type I interferon response

The phosphatidylinositol 3-kinase (PI3K) is activated upon influenza A virus infection in a biphasic manner. An early and transient induction of PI3K signaling is induced by viral attachment to cells and promotes virus entry. In later phases of the infection cycle the kinase is activated by direct interaction with the viral protein NS1, leading to prevention of premature apoptosis. Besides these virus-supporting functions, it was also suggested that PI3K signaling is essential for complete activation of interferon regulatory factor 3 (IRF-3) in response to synthetic dsRNA and IAV infections. However, a direct role of PI3K signaling in the immune response to influenza virus infections was not described yet. Here we show that accumulation of vRNA in human lung epithelial cells infected with either influenza A or B viruses results in PI3K activation. Furthermore, expression of the RNA receptors Rig-I and MDA5 was increased upon stimulation with virion extracted vRNA or IAV infection. Using siRNA approaches, Rig-I was identified as the pathogen receptor necessary for influenza virus vRNA sensing and subsequent PI3K activation in a TRIM25 and MAVS signaling dependent fashion. Rig-I induced PI3K signaling was further shown to be essential for complete IRF-3 activation and consequently induction of the type I interferon response. These data show that PI3K is activated as part of the Rig-I mediated anti-pathogen response to enhance expression of type I interferons.

I03

Analysis of specific amino acids of the NS1 of highly pathogenic avian influenza viruses: Importance for viral replication efficiency

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Keywords: influenza virus, innate immunity, apoptosis

Highly pathogenic avian influenza viruses (HPAIV) with reassorted NS segments from the HPAIV strains A/Goose/Guangdong/1/1996 (GD, H5N1) and A/Mallard/NL/12/2000 (Ma, H7N3) placed in the genetic background of A/FPV/Rostock/34 HAPIV (FPV-H7N1) were generated by reverse genetics. Compared to the wtFPV these reassortants show altered viral replication characteristics. As such, the reassortant FPV-NS-GD shows higher replication rate than the FPV-NS-Ma or the wtFPV. Furthermore, the reassortant viruses show different growth rates and effects on the innate immunity in mammalian cell culture systems. In avian cell cultures all replicate similar: The NS-genes of GD and Ma differ only in 8 amino acids (aa) in the NS1 and 2 aa in the NEP, but the GD- and Ma-NS within the genetic background of FPV show dramatically different effects. We therefore started to analyze which aa or combination of aa in the GD-NS1 would be responsible for the different effects (compared to the Ma-NS); (i) on viral propagation (growth rate) and transmission (host range) and (ii) on the innate immunity and cell-mediated apoptosis. We employ mutational analysis by the generation of recombinant reassortant viruses with mutated Ma-NS segments as well as plasmid based reverse genetic systems. First results will be presented.

I04

Analysis of the antiviral ISG15 system and its role in the replication of human and animal influenza A viruses

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Keywords: ISG15, influenza A virus, species specificity

Influenza A viruses (IAV) can infect a wide range of avian and mammalian species. One important factor determining IAV species specificity is the receptor binding preference. Avian IAV recognize α2,3-linked sialic acids whereas human IAV detect the α2,6-linked form. However, there is still only incomplete knowledge about the virus- and host-encoded factors enabling animal IAV to adapt to the human host concerning transmissibility and pathogenicity. Previous data showed variations in the upregulation of type I interferons and interferon-induced genes like ISG15 after infections with human, avian and porcine IAV. The underlying mechanisms responsible for these different immune responses are subject of our investigation. The ubiquitin-like protein ISG15 can be covalently attached to both host and viral proteins in an enzymatic process mediated mainly by Ube1L, UbcH8 and Herc5. The ISG15 system shows strong antiviral effects, thus several viruses evolved ISG15 evasion mechanisms. We hereby hypothesize that the ISG15 system plays an important role in influenza virus replication, virulence and species specificity. Therefore, we aim to establish targeted gene knockdowns of ISG15, its conjugating enzymes and other innate immune factors in cultured lung epithelial cells and subsequently in an *ex vivo* lung infection model. This lung model was previously set up in collaboration with clinical partners in the context of the BMBF project "FluResearchNet". We will analyze the effects of these knockdowns on infections with human, avian and porcine IAV strains in order to investigate the role of the ISG15 system in limiting IAV species specificity.

I05

The influenza A virus NS1 protein prevents activation of the noncanonical IKK1/p52 NF-κB pathway and limits chemokine expression in lung epithelial cells

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Keywords: influenza A virus, NS1, NF-κB

Activation of NF-κB transcription factors is a major event during infection with many pathogens, including influenza A virus. NF-κB is controlled by two pathways. The classical/canonical pathway proceeds via degradation of IκBs and leads to release of p65-p50 heterodimers. The alternative/noncanonical pathway regulates proteolytic processing of NF-κB2 (p100) to form RelB/p52 dimers. NF-κB and its important role in apoptosis regulation and establishment of innate immune responses upon influenza virus infection has been extensively studied. Sofar the noncanonical pathway has been mainly linked to the regulation of adaptive immunity. Therefore the aim of this study was to elucidate the role of noncanonical NF-κB signaling in influenza virus infected epithelial cells.
Infection with the human H1N1 strain PR8 showed no marked increase of p100 processing or nuclear import of p52/RelB. However a recombinant virus lacking the NS1 protein readily provoked activation of the noncanonical pathway. Transfection of viral RNA also activated the pathway resulting in increased p52 levels. Knock down of the RNA sensor RIG-I prevented activation, suggesting that RIG-I acts upstream of noncanonical signaling. We also investigated influenza induced p52-dependent gene expression. Expression of the chemokine CCL19, a target for p52/RelB was induced by deltaNS1 virus dependent on presence of NF-κB2. The present results indicate that inhibition of noncanonical NF-κB activation by NS1 limits CCL19 expression in epithelial cells. Based on these results we hypothesized that noncanonical NF-κB signaling might be relevant to mount the systemic immune response to influenza virus infection and therefore is strongly antagonized by the viral NS1.

I06

Preclinical evaluation of influenza vaccines based on the replication-deficient vaccinia vector MVA

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Keywords: emerging infections, poxvirus, recombinant vaccine

The zoonotic transmissions of highly pathogenic avian influenza viruses of the H5N1 subtype have sparked the development of novel influenza vaccines.
Modified Vaccinia virus Ankara (MVA), originally developed as a safe smallpox vaccine can be exploited as a viral vector. It has favourable properties, which makes it an attractive candidate as a pandemic influenza vaccine. Recently we have evaluated a MVA-based vaccine for highly pathogenic influenza virus of the H5N1 subtype in mice and macaques. To this end, recombinant MVA was constructed expressing the gene encoding the hemagglutinin of H5N1 influenza virus A/Vietnam/1194/04 (clade 1) (MVA-HA-VN/04) and used to immunize C57BL/6 mice and cynomolgus macaques (macaca fascicularis). Two immunizations induced strong virus specific antibody responses in both species and protected the animals from the development of severe disease observed in control animals inoculated with empty MVA vector or PBS after challenge infection with the homologous or the antigenically distinct influenza virus A/Indonesia/5/05 (clade 2.1). In vaccinated animals virus replication in the respiratory tract was not detectable and the development of histopathological changes in the lungs was prevented. Furthermore, a MVA-based 2009 pandemic H1N1 vaccine protected against severe disease in a pH1N1 ferret model.
The preclinical evaluation of MVA-based candidate vaccines indicated that they have potential as vaccines against highly pathogenic H5N1 and pH1N1 influenza viruses. The MVA-based vaccines proved to be immunogenic and induced broad-protective immune responses. MVA has favourable properties for the production, storage and use as a pandemic influenza vaccine and clinical development seems warranted.

I07

Astrocyte and neuronal gp130 mediated signalling is critical for survival of Toxoplasma encephalitis

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Keywords: *Toxoplasma gondii*, encephalitis, astrocyte, neuron, gp130

Infection of immunocompetent mammals with *T. gondii* induces a chronic infection of astrocytes and neurons. In immunodeficient individuals, the infection can be reactivated resulting in lethal *Toxoplasma* encephalitis (TE), where the parasite is primarily controlled by infiltrating T and B cells, but also brain resident cells contribute to control of the disease. To study these cells in TE, we generated mice with a conditional deletion of gp130 in astrocytes (GFAP-Cre gp130$^{fl/fl}$) and neurons (Synapsin-Cre gp130$^{fl/fl}$).
Infected GFAP-Cre gp130$^{fl/fl}$ mice show a loss of astrocytes in inflammatory lesions, an inefficient containment of inflammatory foci and an impaired parasite control. Mice succumb to TE 50 days after infection, whereas 50% of control mice survive. *In vitro*, infected gp130 deficient astrocytes inhibited the growth of *T. gondii* efficiently after stimulation with IFN-γ, whereas noninfected and TNF-stimulated gp130 deficient astrocytes became apoptotic.
Infected Synapsin-Cre gp130$^{fl/fl}$ mice developed TE with large inflammatory lesions, high numbers of inflammatory leukocytes and increased parasite numbers. Additionally, TE of Synapsin-Cre gp130$^{fl/fl}$ mice resulted in a substantial apoptosis of neurons inside and outside of inflammatory lesions. We observed significantly reduced levels of TGF-ß as well as IL-27. Synapsin-Cre gp130$^{fl/fl}$ animals died significantly earlier in the chronic phase of infection than control mice on day 80 after infection. *In vitro*, gp130 deficient neurons showed an increased apoptosis rate, but no change in invasion and intracellular parasite replication compared to control neurons.
Collectively, these findings demonstrate that astrocyte and neuronal gp130 expression are important protective components of the immune response in TE.

I08

MHC I-antigen processing in Chlamydia-infected dendritic cells

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Dendritic cells (DCs) are likely to be the first professional antigen presenting cells (APCs) that are encountered by chlamydiae during infection. It is thought that cytotoxic T-Lymphocytes (CTLs) primed by infected DCs play an important role in the effective immune response against chlamydial infection. Despite the crucial role of the CTLs, the intracellular pathways of chlamydial antigen presentation by MHC I in infected APCs is still unclear.
We demonstrate that chlamydial infection of DCs leads to morphological DC maturation as well as secretion of different cytokines and chemokines. Chlamydia-infected DCs are additionally characterized by an increased expression of GBP1 suggesting an activation of cell-autonomous resistance mechanisms during infection and DC maturation. Furthermore, we observed at early time points of infection a co-localization of chlamydial vacuoles with late endosomes containing cathepsins, which are believed to play an essential role in the antigen processing of the vacuolar MHC I cross-presentation. Upon chlamydial infection surface presentation of MHC I is up-regulated. This is accompanied by an increased expression of MHC I molecules, which seems to require autocrine TNF-α signaling. Our experiments demonstrate that during DC-infection post-ER MHC I molecules are translocated from the Golgi/TGN to cathepsin-containing compartments. Finally, we have analyzed the stimulation of chlamydia-specific CTLs by infected DCs that are blocked for different components of the classical and non-classical MHC I-machinery. These studies provide the first evidence that chlamydial antigens are processed through a complex intracellular pathway that involves preprocessing in endosomes via different cathepsins and entry into the cytosol for further processing by the proteasome. After antigen export to the cytosol and proteasomal degradation chlamydial antigens

are translocated by the peptide transporter TAP into the ER as well as endosomes and are mainly loaded on MHC I molecules derived from the late secretory and/or endosomal recycling pathway.

I09

Local cellular responses after intrapulmonary inoculation of calves with *Chlamydia psittaci*

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Keywords: calf, *Chlamydia psittaci*, immunity, lung

Experimental intrapulmonary inoculation of calves with *Chlamydia psittaci (C. psittaci)* causes acute pneumonia that mostly resolves within 5 weeks. The objectives of this investigation were to characterize cellular reactions in the lung during acute inflammation and healing. Samples of pulmonary tissue were collected 2, 3, 4, 7, 10, 14 and 35/37 days post inoculation (dpi) from calves that had received 10^8 IFU *C. psittaci* into the lung. Number and distribution of neutrophils, macrophages, dendritic cells, $CD4^+$, $CD8^+$ and $\gamma\delta^+$ T lymphocytes, IgA^+, IgM^+, $IgG1^+$ plasmacells/B lymphocytes as well as chlamydia were examined. Six mock-inoculated calves served as controls. A massive influx of neutrophils into the lung was observed at 2, 3 and 4 dpi and a marked increase of alveolar macrophages by 3 dpi. Foci of necrotic pulmonary tissue were demarcated by macrophages, $CD4^+$ T lymphocytes and dendritic cells at 7 dpi. The extent of pulmonary lesions decreased continuously at 10 and 14 dpi. The remaining lesions that contained chlamydia were infiltrated by macrophages, $CD4^+$, $CD8^+$ and $\gamma\delta^+$ T lymphocytes and demarcated by IgM^+, IgA^+ and IgG_1^+ plasma cells. Multifocal infiltrates of IgM^+ B lymphocytes and $CD4^+$ T lymphocytes were detected along interlobular septae. At 35/37 dpi, most of the lesions had resolved, and number and distribution of immune cells were consistent with the controls. In summary, primary reactions in the lung were characterized by neutrophils, macrophages and dendritic cells. The following influx of cells of the cell-mediated and humoral immune response into the lung coincided with the elimination of chlamydia.

I10

Inoculation with *Chlamydia psittaci* sustainably impairs lung health of calves

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Keywords: *Chlamydia psittaci*, calves, respiratory infection, blood

Chlamydia psittaci (*Cp*) infections can cause acute pneumonia in calves but also are suspected to chronically impair lung functions at a low or sub-clinical level. Our negative controlled study aimed to verify these effects by means of blood analysis and physical examination.

Two groups of animals (n=21 each) were intrabronchially challenged either with 10^8 inclusion-forming units of *Cp* per calf or with BGM cells (control group) diluted in stabilizing medium. Until 35 dpi (days after inoculation) acute-phase proteins LBP (lipopolysaccharide-binding protein) and Hp (haptoglobin) were quantified in blood by ELISA. White blood cell and differential cell counts as well as the clinical score (assessing general condition, respiratory, cardiovascular and other organ systems) were recorded on a daily basis.

Calves responded to *Cp* with a rapid increase of acute-phase proteins LBP and Hp, which returned to baseline within 7-10 dpi. During the acute phase, leukocytes, especially neutrophils, increased massively (unsegmented > segmented granulocytes). In the further course, absolute and relative amounts of segmented granulocytes were elevated compared to controls, while lymphocytes and monocytes only were increased in absolute numbers.

Clinical outcome was dominated by acute respiratory disease (maximum 2-3 dpi), which improved within one week. Compared to controls, an increased respiratory rate, cough and hyperaemic mucous membranes occurred more frequently towards the end of the observation period.

In conclusion, inoculation of *Chlamydia psittaci* in the bovine lung caused acute respiratory illness and systemic host response, but was also capable of inducing a prolonged negative impact on health.

I11

Impaired response of persistent *Chlamydia psittaci* to antimicrobials

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Keywords: *Chlamydia psittaci*, persistence, antimicrobial therapy, rifampicin

Chlamydiae are obligate intracellular pathogens that are able to persist in the host cell, where they enter a viable, but uncultivable aberrant state.
Here we determined the efficacies of several antimicrobials against the zoonotic species *Chlamydia* (*C.*) *psittaci*. The minimal inhibitory concentrations for doxycycline, macrolides, and rifampicin were between 0.016 and 0.08 µg/ ml, those for quinolones were higher.
We established an *in vitro* model of persistence for *C. psittaci* to analyse its sensitivity against several antimicrobials. Doxycycline and erythromycin were significantly less efficient in reducing the number of chlamydiae than their combination with rifampicin. All antimicrobials have not been able to completely eradicate persistent chlamydiae but a reduction of recultivable chlamydiae of more than 99 % could be achieved by early addition of antibiotics.
A real- time PCR- based molecular analysis of antimicrobial treatment of persistent *C. psittaci* revealed different transcription levels of several chlamydial genes in dependence on the applied antimicrobial and the point of time of application. In concordance to the cell- culture assays, doxycycline and erythromycin caused an up- regulation of the examined genes (e.g. *ompA*) whereas rifampicin- treatment led to a down- regulation of *ompA*.
Our experiments show, that an antimicrobial monotherapy of persistent chlamydiae is limited, but that the efficacy can be increased by combination with rifampicin. Real- time PCR can be used as a reliable tool to evaluate the success of a combination therapy of persistent chlamydiae.

I12

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

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Keywords: *Mycobacterium paratuberculosis*, mouse model, colitis, Crohn's disease

Mycobacterium avium subsp. paratuberculosis (MAP) causes so-called Johne's disease (JD), a chronic disease of ruminants with clinical symptoms like severe diarrhea and wasting. Similarity of pathology of Johne's disease and Crohn's disease (CD), a chronic inflammatory bowel disease of humans with clinical symptoms like weight loss and diarrhea suggested a common cause. In fact, MAP has been isolated from the intestine of Crohn's disease patients. Nevertheless, it is still in debate whether MAP is the cause of CD. One reason for this is the absence of suitable mouse models in which the survival of MAP in the host and the raise of inflammation can be followed. Thus far, infection of various mouse strains did not yield concrete results.

Therefore, the hypothesis was derived that MAP is not causing but exacerbating existing inflammatory bowel disease. The aim of this project is to test this hypothesis. Thus colitis will be induced in mice which in turn will be infected by MAP.

In this study, we observed body weight reduction of DSS-induced mice which infected with MAP. After the body weights of the mice were recovered, mice were reinfected with MAP. Interestingly, mice were not only losing their body weight, but also showed diarrhea which are the clinical symptoms of JD and CD. We also observed an increasing weight of spleen and liver at three weeks after infection. Furthermore, we only found viable bacteria in DSS-induced MAP infected mice from gut plating, not in non DSS-induced MAP infected mice at three weeks after infection. These results indicate that the secondary infection of MAP can induce diarrhea and the existing colitis might contribute to the severity of MAP infection.

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I13

The effects of probiotics and zinc supplementation on the porcine innate immune response

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Keywords: *Enterococcus faecium*, Salmonella, PGRPs, NFκB

The aims of this project are to determine the effects of the *E. faecium* NCIMB 10415 probiotic strain and zinc on innate immune responses in swine using a *Salmonella* infection model. Previous *in vitro* studies showed that preincubation of porcine intestinal epithelial cells with cell-free supernatants of *E. faecium* cultures limited the intracellular growth of *Salmonella*. These observations suggested that bacterial components and not the bacteria *per se* were responsible for possible probiotic effects. Chromosomally-integrated NFκB-dependent luciferase reporter fusions in porcine intestinal epithelial and macrophage cell lines have been constructed. High throughput screening of compounds activating or inhibiting NFκB activation is possible. The effects of *E. faecium* and/or components on NFκB activation in *Salmonella* infected cells are being studied.
A second goal is determination of the role of ion aquisation (zinc) on *Salmonella* intracellular survival. *Salmonella* are facultative intracellular bacteria. Following internalization/invasion of host cells, *Salmonella* survive and replicate within a modified phagosome, an intracellular environment where essential elements may not be available. To investigate the relevance of zinc in *Salmonella* intracellular survival, mutant strains have been constructed in which the *zur*, *zraP*, *znuA*, *znuB* and *znuBC* genes have been deleted. *Salmonella* NRAMP homologues (*mntH*)-mutants were also investigated. In contrast to intestinal epithelial cells, the Δ*zur* mutant showed reduced invasiveness compared to the wild-type strain in porcine macrophage cells, although the growth rate during 24 hours is similar. These results suggest that the Zur protein may play a role on early survival in macrophage-like cells.

I14

Antiviral potential of different bacteria species and bacterial metabolites

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Keywords: antiviral effect, bacteriocin, starter cultures

An antiviral effect of bacteriocins as well as bacterial supernatants, e.g. originated from lactic acid bacteria or staphylococci, was described in different studies for example with herpes simplex virus, influenzavirus A and Newcastle disease virus. Application of these bacteria as starter and protective cultures and their metabolites during food processing or as potential probiotics added to food or animal feed can therefore be a promising measure to reduce virus infections in humans and animals. The aim of this research project is to investigate the antiviral potential of different bacteria commonly used as starter and protective cultures during food processing as well as of related bacteriocins.
In first *in-vitro* experiments the antiviral effects of different bacteriocins against enveloped and non-enveloped viruses (influenzavirus H5N6 and H1N1, Feline herpesvirus, Newcastle disease virus and Murine norovirus) were tested. Only for influenzavirus H5N6 a possible antiviral effect of sakacin A and nisin can be supposed. Nevertheless, the virus inactivation by different pH values should be clarified in further experiments. In a second part of this study a variety of culture supernatants of different bacteria species, mainly lactic acid bacteria and staphylococci, was tested for their antiviral activity against enveloped and non-enveloped viruses. Until now only one supernatant of a *Lactobacillus curvatus* strain showed an antiviral effect against the Murine norovirus, widely used as a surrogate for human noroviruses. A selection of relevant bacterial starter and protective cultures will be tested in following experiments for their potential of virus inactivation in raw food products.

Poster Session – Methods and diagnostics

M01

Development of a diagnostic multiplex PCR microarray assay to detect and differentiate *Brucella* spp.

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Keywords: *Brucella* spp., brucellosis, multiplex PCR, microarray

Brucellosis is a worldwide zoonosis leading to tremendous economic losses and severe human illness. Fast and reliable laboratory tests are needed to detect disease in both humans and animals and to monitor the production of safe food products and feed.
For rapid identification of the genus *Brucella* and differentiation of its species, a multiplex PCR microarray assay based on 11 signature sequences and redundant oligonucleotide probes was developed using the easy-to-handle and economical ArrayTube® platform. The gene targets included genus specific sequences in *bcsp31*, *perA*, *cgs*, and *omp2b*, and chromosomal regions displaying hybridization patterns unique for *B. suis* i.e. BR0952, BR0953, BRA0377, BRA0364, specific to *B. abortus* (BruAb2_0168) or *B. melitensis* (BMEI1661 and BMEII0466).
Brucella type and reference strains and a representative panel of 102 field isolates were unambiguously identified by their hybridization patterns. The differentiation of *Brucella* species was limited in members of the two groups *B. suis* bv 3/4/*B. canis* and *B. neotomae*/*B. microti*. The assay specificity was determined to be 100% when testing 31 clinically relevant or closely related non-*Brucella* strains. The detection limit of the *Brucella* ArrayTube® assay was approximately 200 GE.
In summary, the newly developed *Brucella* ArrayTube® assay is an easy-to-handle molecular test for high-throughput and parallel analysis which allows fast response in brucellosis outbreaks.

M02

Establishment of a *Yersinia enterocolitica* infection model in minipigs

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Keywords: *Yersinia enterocolitica*, infection model, swine, minipig

Yersinia (Y.) enterocolitica is the fourth most prevalent bacterial cause of human diarrhea in Germany. Raw pork meat plays an important role as an origin of infection, and swine are considered as a reservoir of human pathogenic Y. enterocolitica. The most prevalent serotype isolated from diseased humans and slaughtered pigs in Europe is the serotype O:3. However, most experimental infection studies have been done in mice, and the few studies in swine used Y. enterocolitica serotype O:8 which is more common in North America. Therefore, we established an infection model for Y. enterocolitica in minipigs for infections with both serotypes O:3 and O:8. Minipigs were selected due to their advantages in handling and housing and owing to their increased use as laboratory animals. Five-week-old piglets were orally infected with Y. enterocolitica serotype O:3 and O:8, respectively. Rectal swabs were taken twice, blood samples once a week. At necropsy intestinal and lymphoid organs were sampled for gross and histological examination. Results revealed that infection could be established with both serotypes, and that serotype O:3 seemed to be more virulent.
In conclusion, we have established a novel infection model in minipigs and found first evidence that serotype O:3 is more virulent in pigs than serotype O:8, which is in contrast to previous reports on experimental infections in mice. Thus, we propose that minipigs might serve as an appropriate model to study pathogenicity and adaptation of zoonotic Yersinia in their natural host reservoir.

M03

Towards a novel screening approach for hepatitis E virus antibodies in pigs

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Keywords: Hepatitis E virus, pigs, antibody assay, meat juice

Hepatitis E virus (HEV) is the causative agent of an acute, self-limiting hepatitis and is responsible for water-borne outbreaks in developing countries. The fact that HEV infections in Europe were recently observed in individuals without a travel history led to the assumption of an animal reservoir. Indeed, earlier studies revealed that HEV is endemic in the domestic pig population in Germany [1]. Phylogenetic studies confirm porcine HEV being closely related to human HEV variants and rising figures of notified clinical hepatitis E cases give concern to increased zoonotic transfer of HEV.
In order to reduce the risk of HEV infections either of food-borne origin or through direct contact to infected animals, identification and monitoring of HEV-positive pig herds is essential. Regarding individual samples, serological testing is less expensive than diagnosis based on nucleic acid amplification. Apart from porcine serum samples, porcine meat juice constitutes a promising target for monitoring HEV infections of domestic pigs. Meat juice is routinely used for testing pig herds on *Salmonella* infections and should be considered for investigation of further infectious diseases as well [2]. On the way to a novel antibody assay, working steps will include HEV antigen expression, antibody-ELISA development and diagnostic screening of pig herds to gain better knowledge on the epidemiology of HEV in Germany.

[1] Baechlein, C., Schielke, A., Johne, R., Ulrich, R. G., Baumgärtner, W., Grummer, B., 2010. Prevalence of Hepatitis E virus-specific antibodies in sera of German domestic pigs estimated by using different assays. Vet Microbiol. 144, 187-191.
[2] Blaha, T., Klein, G., Nobmann, J., Meemken, D., 2010. Monitoring via „meat juice serology". Proc. 2nd European Symposium on Porcine Health, Hannover

M04

Development of real-time RT-PCR assays for the detection of potentially zoonotic viruses in faeces of pigs

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Keywords: porcine viruses, real-time RT-PCR

Several viruses closely related to human viruses could be detected in faeces of pigs. A transmission of these viruses to humans and a potential for causing human disease may be therefore suspected. Little is known about the prevalence of such potentially zoonotic viruses in pigs like astrovirus (AstV), encephalomyocarditis virus (EMCV), hepatitis E virus (HEV), norovirus (NoV), rotavirus group A, B and C (GARV, GBRV and GCRV) and sapovirus (SaV). Several RT-PCR protocols for the detection of these viruses have been described, but only few or no real-time RT-PCR assays are available. The aim of our study was to establish real-time RT-PCR assays for sensitive and quantitative detection of these viruses.

For the development of real-time RT-PCR assays, multiple alignments with sequences from GenBank database were performed and primers and probes were designed. To enable an absolute quantification, RNA-standards were prepared by cloning PCR-products and subsequent *in vitro* transcription, and standard curves were generated. Real-time RT-PCR assays for the detection of AstV, EMCV, HEV, NoV and GARV were established. The protocols show PCR-efficiencies between 90% and 100% and minimum detection limits between 14 and 65 viral copies per reaction. Development of real-time RT-PCR assays for the detection of GBRV, GCRV and SaV is in progress.

Application of the quantitative real-time RT-PCR assays will enable determination of the prevalence of potentially zoonotic viruses in pigs. Hereby, a risk of zoonotic transmission to exposed groups like farmers or veterinarians, as well as to the consumer, can be evaluated.

M05

Identification of specific epitopes for 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 and is transmitted by mosquitoes. The insect vectors can also infect birds and mammals such as horses or humans. However, the viremia in infected mammals is not high enough to allow further transmission of WNV, hence mammals are considered dead-end hosts for WNV. Nevertheless, an infection can lead to severe neurological disease. Specific serologic diagnosis of WNV infection is hampered by the fact that antibodies to WNV-structural proteins display considerable cross-reactivity to other flaviviruses. To identify new specific, non-crossreactive epitopes, we are executing a serologic screen on the WNV-proteome, using short bacterially expressed protein-fragments. These proteins are coated on microtitre plates and are incubated with several human and animal sera from individuals infected with WNV or other flaviviruses.

M06

Identification of RNA- and DNA-viruses in CSF of patients with aseptic meningo-encephalitis of unknown ethiology via pyrosequencing

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Keywords: meningo-encephalitis, CSF-pyrosequencing, meta-genome analysis

The ethiology of more than 50% of all aseptic meningo-encephalitis cases is unclear. A part of these diseases could be caused by neglected zoonotic arboviruses for which no diagnostic methods are available. There is also the possibility that new, as yet unidentified viruses or microorganisms are responsible for the development of the disease. The new technique of next-generation sequencing (NGS) presents an unprecedented opportunity to identify new pathogens in so far idiopathic diseases. The goal of this pilot project will be to perform a metagenome analysis of 88 clinically well characterised CSF samples with unknown ethiology to identify new pathogens that can cause meningo-encephalitis. Compared to serum samples, CSF samples have the advantage that they posses a lower amount of human cells that can lead to contaminating sequences. Recently we were able to decipher the complete genomes of seven RNA-viruses and one DNA-virus from cell-culture supernatants with high coverage via 454 pyrosequencing. Here we already optimized protocols for the enrichment of viral particles and reduction of contaminating sequences. The results could help to develop new diagnostic tools for the identified microorganisms and could give rise to prevalence surveys and further characterization of these organisms.

M07

Development, establishment and validation of real-time RT-PCR assays for the detection and characterisation of Lyssaviruses

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Keywords: Lyssavirus, rabies, real-time RT-PCR

Lyssaviruses belong to the family *Rhabdoviridae* within the order *Mononegavirales*. These are single stranded RNA viruses with a negative sense genome. The Lyssavirus genus within the family *Rhabdoviridae* is separated into virus species, namely Rabies virus (RABV), Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV), European bat lyssaviruses type 1 and 2 (EBLV-1 & -2), Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV), and West Caucasian bat virus (WCBV). New species like Shimoni bat virus (SHIBV) and Bokeloh bat lyssavirus (BBLV) has recently been isolated from lesser horseshoe bat (*Hipposideros commersoni*) and Natterer's bat (*Myotis nattereri*), respectively. Although dog transmitted rabies causes the majority of the estimated 55,000 casualties per year, most bat lyssaviruses have also caused human diseases. A high degree of genetic variability of lyssaviruses can be ascertained based on the broad range of hosts, the geographical distribution and the evolutionary age. Thus the development of real-time RT-PCR assays for the reliable detection and characterisation of all lyssaviruses is crucial for the fight against rabies and rabies-like diseases in Europe and worldwide.
With this approach different pan-lyssavirus PCR systems for the identification of a wide range of lyssaviruses were established as well as newly developed. Well-established primers of the conserved N gene were used for the set up of two SybrGreen-based real-time RT-PCR assays. Furthermore a new pan-lyssavirus real-time RT-PCR assay using conserved primer-binding sides of the polymerase (L) gene was developed. For the validation of all three assays a representative panel of lyssaviruses, including RABV, MOKV, EBLV 1 and 2 as well as BBLV, was used. The analytical and diagnostic sensitivity of the different assays were optimized using two-step and one-step RT-

PCR approaches. The one-step RT-PCR assays clearly showed a better sensitivity and furthermore the one-step protocols reduce the assay time and prevent cross-contaminations. For the characterization of pan-lyssavirus genome positive samples the real-time RT-PCR fragments were purified and analysed by a pyro-sequencing method.

M08

Lyssavirus whole genome sequencing

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Keywords: Lyssavirus, sequencing, deep sequencing, Genome Sequencer FLX

Rabies is a worldwide viral zoonosis caused by lyssaviruses of the family *Rhabdoviridae* order *Mononegavirales*. With an estimated 50.000 annual human cases 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 frame 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.
One main aspect of individual project 4 is to establish sensitive and reliable methods for lyssavirus whole genome sequencing. A central issue in virus sequencing in general is to focus the sequencing efforts to the target nucleic acids of the virus and not those of the host. To this end, different methods for preparation of DNA suitable for sequencing are compared. In particular, preparation of DNA using a random primed cDNA synthesis and a targeted approach utilizing primers designed to bind conserved regions of the genome are adopted. Application of random shotgun protocols requires higher amounts and purity of viral RNA, therefore different enrichment techniques are tested. With regard to targeted cDNA synthesis methods, different approaches are evaluated. On the one hand, we examine a combination of multiple PCRs and Sanger sequencing, on the other hand, fewer but longer PCR products are used as input for sequencing on the Genome Sequencer FLX. To test the reliability of the different methods, the quality of the final sequences is compared.

M09

Analyses of the genome diversity of Lyssaviruses using the genome sequencer FLX

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Keywords: in depth sequence analysis, structural variant, lyssavirus, Genome Sequencer FLX

Lyssaviruses are long known to produce different amounts of defective interfering (DI) particles. These DI particles have incomplete genomes which are structural variants of the complete genome. Previous analyses of genomes from DI particles relied on cloning of the variant genomes from DI particles prior to Sanger sequencing and structural analysis. During this cloning step, genome variants with lower abundance may be depleted and therefore are not available for subsequent sequence analyses. Hence, information about the full genome diversity found in a certain virus population may be lost. With the advent of next generation sequencing technologies, unbiased sequencing of DNA and RNA became feasible. Due to the tremendous sequence depth that can now be achieved paired with the unbiased template preparation, investigations even of low abundance structural variants of virus genomes became easily possible. Here, I present data from a pilot study of genomic variation found in the raw sequence data of three different rabies virus populations. First, the variant genomes found in the different populations are analysed qualitatively. Moreover, the concordances and differences in the structural variants of the three viruses are compared. In addition, the relative abundances of the different variants can be approximated from the available raw sequencing data. In depth analysis of the variants occurring in different virus populations may provide further input to functional virus analysis.

M10

Detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in jejunal and ileocaecal lymphnodes of naturally infected calves

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Keywords: paratuberculosis, calves, lymph nodes, Real Time-PCR

Paratuberculosis is a chronic, untreatable intestinal inflammation of ruminants. Additionally, the causal agent *Mycobacterium avium* ssp. *paratuberculosis* (MAP) has been suspected since years to be involved on the development and/or maintenance of the human disease known as Crohn's Disease. The cattle become infected intrauterine or in the first weeks or months of their life by ingestion of MAP from colostrum, milk, environment or contaminated utensils; but 18 to 20 months could pass until the disease breaks out. Up to now, only diagnosis for infected animals older than two years is successful. The aim of our project "Early diagnostics of MAP infections in cattles" was to develop a method for early detection of young subclinical infected animals. In the first phase of the project, a Real Time-PCR (Schönenbrücher et al., 2008) for detection of MAP in biopsies of jejunal and ileocaecal lymph nodes was established in an animal experiment. In the second phase of the project, the method developed in the infection model, was used on 32 calves from herds with known paratuberculosis history, and on 15 calves from paratuberculosis free herds. Results show that the detection of MAP in jejunal and ileocaecal lymph nodes is also successful on naturally infected calves from an age of 3 months onwards. For this, the Real Time-PCR can be recommended as a sensitive, cost- and time-saving method to detect MAP in calves. The project is funded by the „Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz" and the „Bundesanstalt für Landwirtschaft und Ernährung" (support code: 28-1-32.006-06).

M11

Formation and detection of cell wall deficient forms (spheroplasts) of *Mycobacterium smegmatis* as a model for *Mycobacterium avium* ssp. *paratuberculosis* (MAP)

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

Mycobacterium smegmatis (*M. smegmatis*) is a fast-growing, non-pathogenic microorganism of the family *Mycobacteriaceae*. Due to the short generation time, *M. smegmatis* is frequently used as a surrogate for *Mycobacterium avium* ssp. *paratuberculosis* (MAP), a slow-growing and pathogenic mycobacterium. An infection with MAP causes a chronic, incurable inflammation of the intestinal tract in ruminants (paratuberculosis).
Since the mid-twentieth century, there is a controversial discussion whether MAP is involved in Crohn's disease – a chronic bowel inflammation in humans. MAP has already been isolated from intestinal tissue and blood of patients with Crohn's disease.
The question whether cell wall deficient cells of MAP are participated in the etiology of Crohn's disease is also disputed. Up to now, spheroplasts of MAP have been isolated from resected intestinal tissues of patients with Crohn's disease. Spheroplasts might not be detected by human immune system and by common investigation methods because of their modified cell wall. Therefore the detection of spheroplasts is difficult and time-consuming.
This article presents the formation of cell wall deficient forms of *M. smegmatis* and their detection *in vitro* using culture and microscopic investigation methods (light, fluorescence, electronic microscopy). Furthermore, the conversion of spheroplasts into cell wall competent forms was investigated, wherefore several media were compared.
The formation and detection of cell wall deficient forms und the regeneration to cell wall competent forms of *M. smegmatis* serve

as a model for future researches of MAP. Hereby, new insights could be obtained due to the zoonotic potential of MAP.

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M12

Identification and differentiation of *Toxoplasma gondii* infections by using recombinant antigens in a lineblot assay

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Toxoplasma gondii is one of the most successful protozoan parasites with a broad host range and cats as primary host. Within the alternate hosts only the asexual stages are present. Thereby, tachyzoites are predominantly found in an acute infection while cysts containing bradyzoites are present during the chronic phase of infection. In both infection stages the parasite expresses stage-specific antigens.
We tried to identify *Toxoplasma* antigens that might be suitable for the serological identification of (i) a *T. gondii* infection and (ii) to discriminate an acute from a chronic infection.
By using 2D gel electrophoresis and mass spectrometry we could identify possible markers for the acute stage. Afterwards, five proteins identified by mass spectrometry analysis as well as seven other proteins were recombinantly expressed in *E. coli*. Furthermore, their diagnostic qualities were examined in a lineblot assay with human serum samples. Thereby, a *Toxoplasma* infection could be recognized in up to 100 % by detection of GRA1, GRA2 and GRA6 specific IgG antibodies. However, IgG antibodies against these antigens did not allow the discrimination between the infection stages. The additional detection of GRA6-specific IgA and SUB1-specific IgG antibodies enables the identification of the acute infection with the likelihood of up to 92 %.

M13

Toxoplasma gondii in turkeys - an examination method for large samples

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Keywords: *Toxoplasma gondii,* Sequence capture PCR, magnetic beads, turkey meat

Toxoplasma gondii is one of the most common zoonotic parasites worldwide. An infection can cause serious damage to foetuses, when the mother is first infected during pregnancy. Also immunocompromised humans are at risk for a serious outcome.
Infection of humans mainly occurs by two routes. On the one hand, the parasite can be taken up via direct contact with oocyst-containing cat faeces. On the other hand, consumption of raw or not sufficiently heated, tissue cyst-containing meat from infected food-associated animals can lead to an infection.
A previous study showed a seroprevalence of 18.4 % in German turkeys. For the detection of *T. gondii* in turkey meat by means of quantitative real-time PCR only small sample sizes of 25 mg could be analysed. But since the parasites aggregate in tissue cysts, which are not evenly distributed in the muscle tissues, the detection was hindered.
To overcome this problem and to assess the risk of turkey meat being a source of infection for humans, larger sample sizes should be analysed. This can be accomplished by means of a sequence capture PCR that uses specific biotin-labelled oligonucleotides and streptavidin-coupled magnetic beads to extract the *T. gondii* target sequence out of a 100 g sample. That target sequence can then be amplified by real-time PCR.

M14

Analysis of clonal type-specific antibody reactions in *Toxoplasma gondii* seropositive humans from Germany by peptide microarray

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Keywords: *Toxoplasma gondii*, toxoplasmosis, peptide microarray, serotyping

Toxoplasma gondii has a clonal population structure with clonal types I, II and III dominating in Europe. There are indications that these clonal types are associated with different clinical manifestations of toxoplasmosis in humans and animals. To obtain insights into the clonal types of *T. gondii* in Germany and to explore peptide spectra recognized by human sera, a peptide-microarray test was established using 56 synthetic peptides, which mimic clonal type-specific epitopes. Human sera (n=174) were collected from seropositive patients with acute signs of *T. gondii* infection (n=21), patients with latent *T. gondii* infection (n=53) and from *T. gondii*-seropositive forest workers (n=100). The majority (n=124; 71%) of all *T. gondii*-positive human sera showed reactions against synthetic peptides with sequences specific for clonal type II (type II peptides). Type I and type III peptides were recognized by 42% (n=73) or 16% (n=28) of the human sera, respectively, while type II-III or type I-III peptides were recognized by 49% (n=85) or 35% (n=62) of sera, respectively. A proportion of 13% (n=22) of the sera showed no reaction with type-specific peptides. The results of our study suggest that humans in Germany are

predominantly infected by *T. gondii* of clonal type II and that infections with *T. gondii* of clonal types I or III are less prevalent.

M15

Identification of oocyst specific antigens of *Toxoplasma gondii*

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Keywords: *Toxoplasma gondii*, oocyst, antigen, Western blot, IFAT

To assess the relative importance of different *T. gondii* infection routes, serological tests are needed which can differentiate between *T. gondii* infection caused by the ingestion of oocysts or by tachyzoites/bradyzoites. Therefore we aimed at the identification of antigens specific for the oocyst stage of *T. gondii*. To this end, we immunized two groups of BALB/c mice with *T. gondii* oocyst antigens, soluble or insoluble in water, to generate a set of oocyst-specific hybridoma clones. Screening hybridoma cell culture supernatants by Western blot using oocyst antigens revealed three monoclonal antibodies (MAbs) D3/9, K3/7-13 and 1/6-15-2, which recognized oocyst antigens with approximate molecular weights of 27 kDa, 30 kDa or 65/170 kDa (double band), respectively. IFAT using oocysts, sporocysts and sporozoites as antigen showed K3/7-13 and 1/6-15-2 reacting with the inner layer of oocyst and sporocyst walls. MAB D3/9 exclusively reacted with sporozoites. By means of sodium meta-periodate treatment, carbohydrate residues of the antigens were oxidized, allowing to assess the impact of these residues on epitope binding. We observed a reduction of signal strength in Western blot after sodium meta-periodate treatment for D3/9, K3/7-13 and 1/6-15-2 of about 50%, 10% and 60%, respectively. Western blot analysis and immunofluorescent antibody test (IFAT) revealed that two MAbs, K3/7-13 and 1/6-15-2, reacted faintly also with epitopes in *T. gondii* tachyzoite antigen, whereas D3/9 showed no reaction. Future studies aim at the chromatographic isolation and further characterization of these antigens and at the validation of these antigens for diagnostic purposes.

M16

Sequencing of NTNH genes shows higher conservation than corresponding BoNT genes in *Clostridium botulinum*

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Keywords: *Clostridium botulinum*, botulinum neurotoxin, non-toxic non-hemagglutinin, gene cluster

Botulinum neurotoxins (BoNTs) are secreted by different *Clostridium* species in association with non-toxic complex proteins and are the causative agents of botulism, a severe paralytic illness in men and animals. The genes encoding BoNT and the complex proteins are arranged in a cluster, which has been shown to exist in two different forms: *ha* cluster and *orfX* cluster. In both cluster types the gene for the non-toxic non-hemagglutinin (NTNH) is located immediately upstream of the BoNT gene.
To complement our sequence information of the BoNT genes of our strain collection, we established a PCR using two sets of partially degenerated primers to sequence the NTNH gene of *ha* and *orfX* clusters from *Clostridium botulinum* BoNT/A, /B, /E and /F strains. Comparison of the obtained NTNH sequences with the BoNT sequences of these strains confirms that NTNH genes are more conserved than the corresponding BoNT genes.

M17

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

Based on the complex structure of botulinum neurotoxins (BoNT) and their high toxicity diagnostics of botulism is 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 0.1 ng/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 to 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.

M18

Sensitive and specific sandwich-ELISA for botulinum neurotoxin serotype C and D

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Keywords: Botulinum neurotoxin, sandwich ELISA

Botulinum neurotoxins (BoNT) produced by *Clostridium botulinum* are known as causative agents of botulism, a severe paralytic illness in men and animals. Whereas BoNT serotypes A, B, E and F are associated with human disease, BoNT/C and /D cause botulism in animals (e.g. cattle, poultry). To date in the majority of veterinary cases botulism is diagnosed by mouse bioassay, observing typical symptoms and disease progression. BoNTs are known as most toxic substances on earth and have to be detected from complex matrices like feces or stomach contents; therefore, the setup of highly specific and sensitive immunological assays is challenging keeping in mind that cross-reactivities have to be excluded. Here, we generated monoclonal and polyclonal antibodies in mice and chicken, respectively, using non-toxic recombinant variants of BoNT/C and /D for immunization. With these reagents we established two sandwich ELISA which specifically detect BoNT/C and BoNT/D within the pg/mL-range. The assays are a promising tool for future diagnostic applications in the field of veterinary medicine.

M19

Higher drug concentrations are necessary for the use of oseltamivir as control compound in antiviral studies with pandemic influenza A (H1N1) 2009 viruses in mice

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Keywords: oseltamivir, pandemic influenza, antiviral mouse model

In 2009 pandemic H1N1 influenza A viruses (H1N1v) emerged that contain the NA and M gene segment of Eurasian swine influenza A viruses (sFLUAV). By donating these genes, sFLUAV conferred ion channel inhibitor resistance and neuraminidase inhibitor (NAI) susceptibility to H1N1v. These recent developments prompted us to establish mouse models with currently circulating H1N1v and oseltamivir as control compound for antiviral studies.
Initially, pathogenicity of 3 German H1N1v that are highly NAI susceptible in a chemiluminescence-based enzyme inhibition assay was compared in BALB/c mice.
To establish the antiviral mouse models, lung isolates of the 2 most pathogenic H1N1v were used to intranasally infect BALB/c mice. Oseltamivir was administered orally at 10 mg/kg/d, d 0-4, twice daily. The H1N1v induced a severe disease leading to maximum 20 or 100% mortality in placebo- as well as oseltamivir-treated mice. Moreover, no therapeutic effect was observed in regard on body weight and clinical score monitored till day 21 p. i., lung weight, virus load in lung tissue, and lung histopathology. To test whether a higher oseltamivir dose will result in a therapeutic effect, mice infected with the most pathogenic H1N1v were treated with 100 mg/kg/d of oseltamivir. In the result incomplete reduction of mortality, significant less body weight loss, improvement of general condition of survivors, and reduced virus load in lung tissues was observed. In conclusion, higher drug concentrations as well as prolonged drug administration seems to be necessary for use of oseltamivir as control compound in antiviral studies with H1N1v in mice.

Poster Session – Pathogen-cell interaction

P01

Modulation of gene expression in orthopoxvirus infected cells

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Orthopoxviruses can cause disease in numerous host species including humans. Some orthopoxviruses also have the ability to infect non-natural host species and to cause zoonoses. For instance, cowpox virus and closely related vaccinia virus are both capable of establishing infection in various mammals, including humans. However, infection with vaccinia virus causes a milder disease with a reduced inflammatory response. These differences may be explained by the unique repertoire of host cell modulating factors encoded by vaccinia virus and cowpox virus, allowing productive replication in specific cells and tissues and influencing general pathogenesis.
We aimed at characterizing the specific modulation of the host cells gene expression profile by orthopoxvirus infection. In our study we analyzed changes in host cell gene expression of HeLa cells after infection with cowpox virus or vaccinia virus and compared these to each other and to the gene expression profile of non-infected cells using Agilent Whole Genome Microarray technology. Changes in the expression of selected genes were afterwards verified by TaqMan quantitative real time PCR.
We could identify major differences in chemokine gene expression in cowpox virus and vaccinia virus infected HeLa cells. Furthermore, strong induction of IL-6, IL-8 and CXCL1 secretion was identified in the cell culture supernatant following infection with cowpox virus but not after infection with vaccinia virus.
The observed differences may contribute to the greater inflammatory response towards cowpox virus infection compared to vaccinia virus infection in certain host species.

P02

Proteome analysis of Vaccinia virus IHD-W infected HEK 293 cells with 2-dimensional gel electrophoresis and MALDI-PSD-TOF MS of on solid phase support N-terminally sulfonated peptides

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Keywords: viral proteomics, Vaccinia virus, MALDI-PSD-TOF MS, SPITC

Despite the successful eradication of smallpox by the WHO-led vaccination program, poxvirus infections continue to present a considerable health threat. The possible use of smallpox as a bioterrorist threat as well as the continuous occurrence of zoonotic poxvirus infections document the relevance to deepen the understanding of virus–host interactions. Since the permissiveness of cells for poxvirus infections is independent of cell surface receptors, but correlates with the ability of the virus to infiltrate the antiviral host response, successful infection directly depends on the host's proteome set. In this study the proteome of HEK293 cells infected with Vaccinia Virus IHD-W was analyzed by 2-dimensional gel electrophoresis and MALDI-PSD-TOF MS in a bottom-up approach. The modulated expression of 24 human proteins by the infection was identified from post source decay spectra of N-terminally sulfonated peptides. The proteome analysis of infected cells provides insights into apoptosis modulation, regulation of cellular gene expression and the regulation of energy metabolism. Several of the regulated human proteins have not yet been described in correlation with poxvirus infections.

P03

Transcriptome analysis of cowpoxvirus infected cells: Comparison of wild-type virus and a mutant lacking the host range factor CP77

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Keywords: cowpoxvirus, host range, CP77, transcriptome

Apart from the threat of a bioterror attack using smallpox, re-emergence of orthopoxviruses in the human population through zoonotic infections is observed. Outbreaks like buffalopox in India, monkeypox in Africa or cowpox in Europe raise concern to the unvaccinated population and emphasize the importance of understanding host range mechanisms. The tropism of a poxvirus species is highly dependent on its unique repertoire of expressed host range genes that interact with the infected cell. Cowpoxvirus (CPXV) host range factor CP77 was identified to be required for replication in Chinese hamster ovary (CHO) cells, but the molecular mechanism by which CP77 modulates host range function remains poorly understood.

For the elucidation of molecular events that determine whether virus replication in CHO cells is abortive or permissive, a CPXVΔCP77 deletion mutant was constructed using bacterial artificial chromosome (BAC)-technology. CHO cells were infected with either CPXVΔCP77 or wild-type CPXV, cells were harvested and total RNA was isolated. Deep sequencing-based transcriptome analysis was performed using Illumina mRNASeq technology, revealing genes expressed differentially in cells possessing or lacking the essential viral host range factor CP77. Further analysis of the involved signalling pathways will help understand the molecular mechanism of CPXV host range.

P04

The importance of TRAIL/FasL for the caspase-dependent nuclear RNP export in influenza virus-infected cells

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Keywords: TRAIL/FasL, caspase, nuclear pore complex, ribonucleoprotein complex

Previous work has shown that the influenza virus (IV)-induced apoptotic caspase activity, which seams to be activated by pro-apoptotic factors such as TRAIL/Fas, promotes nuclear ribonucleoprotein (RNP) export in the late phase of the IV replication cycle. We want to examine in detail the molecular mechanisms and factors involved. Therefore, we have started to investigate to what extent TRAIL/FasL affect the caspase-dependent nuclear RNP export in IV-infected cells. Caspase-activity can degrade the nuclear pore complex (NPC). To this point, we furthermore started to analyzed whether this also takes place in the IV-infected cells. Because it is known that nuclear RNP export is also CRM1-dependent, we have analyzed whether at a certain time point post infection RNP-export switches from active transport to caspase-dependent nuclear evasion and whether this is regulated by TRAIL/FasL. As IV-induced apoptosis, which affects RNP export could be caused by death-ligand mediated signalling our investigation will allow correlating TRAIL/FasL-signalling with the viral replication cycle. First results will be presented.

P05

Interference of influenza viruses and *Staphylococcus aureus* on the level of cellular signal transduction processes (Pilot project: P-16)

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Keywords: Influenza virus, Staphylococcus aureus, pneumoniae, signal-transduction

Influenza A viruses (IAV) and *Staphylococcus aureus* belong to the most harmful pathogens inducing respiratory diseases, which are often associated with necrotising pneumonia in humans. Co-infections with both pathogens are barely understood, difficult to treat and characterised by high mortality rates.
Upon viral or bacterial infections the invading pathogens are detected via pathogen recognition receptors, concurrently inducing cytokine and chemokine expression, inflammation, apoptosis, and/or necrosis. In turn the invaders have developed strategies to counteract the cellular defence mechanisms. Current scientific knowledge strongly indicates an interference of both pathogens on the level of signal transduction processes. Although, cellular signalling induced by either IAV- or *S.aureus* is quite well characterised, signal transduction processes, which are induced upon co-infections with both pathogens are only marginally described. We will exemplarily investigate the interference of IAV and *S.aureus* during development of infections in cell culture systems. The aim of the study is to identify cellular targets that are manipulated by IAV and *S.aureus* for their own benefit, to develop novel antiviral/antibacterial strategies that ensure an effective treatment of both. Therefore we will establish infection models in cell culture to investigate IAV-induced signal transduction in the presence of bacterial toxins (e.g. Panton-Valentine Leukocidin) and wall components (e.g. lipoteichoic acid) of *S.aureus.* Afterwards we will interfere with co-induced cellular signalling via different biochemical methods to determine the functional contribution to viral and bacterial propagation and to characterize the cellular effects.

Our studies will also pave the way for further investigations of other zoonotic co-infections, bridging not only virology and bacteriology but also human and veterinary medicine.

P06

Recombinant soluble influenza A hemagglutinins bind to cell surface sialic acids of cultured cells and tissue sections

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Keywords: influenza, soluble hemagglutinin, sialic acids, receptor-binding

The interaction of influenza viruses with host cells is mediated by the hemagglutinin (HA). This viral surface glycoprotein recognizes sialic acid residues as a receptor determinant. Most avian influenza viruses preferentially bind to α2,3-linked sialic acids whereas human viruses prefer α2,6-linked sialic acids. For an avian virus to infect human cells a change in receptor specificity is therefore needed. To distinguish between these two linkages, the lectins MAA and SNA were used in many studies. Due to the huge diversity of oligosaccharides and the different HA subtypes, the two plant lectins are not sufficient to characterize the cellular ligands recognized by influenza HA.

We use soluble recombinant HAs of different subtypes in comparison with the classic lectin stains to investigate receptor distribution on different cell lines and sections of avian trachea and porcine lung.
The binding pattern and density differs between hemagglutinins of the H7 and the H9 subtypes. Comparison of binding of soluble HAs and lectins shows that the latter does not necessarily correlate with the expected binding preference of the subtype, i.e. weak H7 binding despite a strong MAAII staining. Prominent binding of the soluble hemagglutinins was also detected to the differentiated epithelium of the chicken and turkey trachea, with the H9 showing a weaker binding compared to the H7 protein.

These experiments show that a plant lectin staining alone cannot sufficiently characterize the distribution of the cellular interaction partners for influenza A viruses. Soluble hemagglutinins promise to be a valuable tool to visualize potential influenza binding sites on cells and tissues.

P07

Relevance of Raf/MEK/ERK-cascade and PI3-cascade for influenza virus RNP-function and localization

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Keywords: Influenza Virus, Nucleocapsidprotein, Raf/MEK/ERK-cascade

Influenza viruses (IV) pursue a nuclear replication strategy. After virus entry the viral ribonucleoproteins (RNPs) are transported into the nucleus, were replication and transcription of the viral genome takes place. Later when sufficient viral proteins have been produced and new RNPs have been generated these have to be transported out of the nucleus to be enwrapped into progeny virions that bud from the cell surface. We have previously shown that IV activates Raf/MEK/ERK-signaling late in the replication cycle, which is an essential prerequisite for efficient nuclear RNP-export. The nucleocapsid protein (NP) is a major component of the RNPs and is a phospho-protein. Cellular localization and thereby function seems to be affected by phosphorylation. In addition it is known that RSK, which is activated downstream of Raf/MEK/ERK-signaling, can phosphorylate RanBP3, which can modulate the Ran gradient between nucleus and cytoplasm and thereby the cellular nucleo/cytoplasmatic transport. We have determined the relevance of virus-induced ERK and RSK activity for NP phosphorylation and its impact on the nuclear RNP export. In order to elucidate whether virus-induced ERK activity and/or RSK activity affect NP/RNP localization/function, infected cells were treated either with or without a MEK and/or RSK-Inhibitor. NP was isolated and phosphorylation was analyzed by LC/MS/MS. Affected amino acids were mutated and NP localisation and function was investigated in plasmid based replication system.

P08

Influenza A virus host adaptive mutations in PB2 D701N and NP N319K mediate enhanced tissue tropism and immune cell depletion in mice

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Keywords: influenza A, polymerase, lymphocyte depletion, pathology

We have previously shown that a highly pathogenic avian influenza virus (SC35; H7N7) predominantly infects the alveolar epithelium accompanied by a mild bronchiolitis and low pathogenicity for mice. In contrast, its mouse-adapted variant (SC35M; H7N7) preferentially infects the bronchial epithelium as well as a broad range of immune cells resulting in T-cell depletion and high virulence in mice (Gabriel et al., 2009).
In this study, we have analysed the role of single substitutions between SC35 and SC35M in cell tropism and immune cell regulation.
We found that single mutations in PB2 (D701N) and NP (N319K) mediate a switch from alveolar to bronchial infection of the murine lung epithelium. Further, these mutations were sufficient to cause severe T-cell depletion similar to SC35M in mice.
Taken together, these data suggest that mutations in PB2 and NP play a crucial role in enhanced tissue tropism and regulation of the host immune response.

P9

First insights in cellular signaling regulated by co-infection/ stimulation of influenza A viruses and lipoteichoic acid from *Staphylococcus aureus*

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Keywords: influenza virus, *Staphylococcus aureus*, pneumonia, signal-transduction, lipoteichoic acid

Influenza A viruses (IAV) are the causative agents of severe respiratory diseases, often accompanied by a high mortality rate. The majority of fatal outcomes are linked to secondary bacterial pneumonia, mainly caused by Staphylococcus aureus (S.aureus). Despite the knowledge of this correlation, little is known about the molecular mechanisms, especially about the cellular signaling events that might be altered due to the interplay of these pathogens. This prompted us to investigate the cellular mechanisms, which are regulated by IAV and S.aureus.
We first assessed how a specific bacterial compound, lipoteichoic acid (LTA), a major constituent of the cell wall of S.aureus, may interfere with IAV-induced signaling. This amphiphilic protein has been shown to activate NF-κB and MAP kinases (MAPK) as well as the phosphatidylinositol-3 kinase signaling cascade. These pathways are also modulated during IAV infection.
First results indicate an increased activation of the MAPK ERK2 and an enhanced expression of the inflammatory mediator Cyclooxygenase-2 (COX2) after LTA stimulation in vitro. Similar results are described in presence of IAV infection. However, co-stimulation of IAV-infected cells with LTA results in decreased, rather than enhanced ERK2 activation and COX2 expression. These results indicate a differential regulation of cellular signaling pathways, which are induced upon IAV and LTA challenge. We aim to provide deeper insights into the regulation of mRNA and protein expression, as well as protein activation, influenced by co-stimulation with IAV and LTA to elucidate the interplay of these pathogens on cellular level.

P10

Neuropathogenicity of TBE viruses in human cell culture *in vitro* models

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Keywords: *Flaviviridae*, TBEV

Tick-borne encephalitis virus (TBEV), a member of the family *Flaviviridae*, causes one of the most important inflammatory diseases of the central nervous system in Europe. The tick-borne encephalitis (TBE) in Germany is the most important arthropod-borne virus infection. Despite the strong awareness of the disease only a few TBEV strains were isolated, characterized and compared.The knowledge on the virulence of the virus strains and their effect on neuronal cells are poorly understood so far.
The aim of the proposed study is the isolation of TBEV from ticks, their sub typing and molecular and cell-biological characterization. As an *in vitro* model infection in human neuroblastoma (SIMA) and glioblastoma cells (DBTRG) was established and used to test the replication competence of different TBEV strains and mechanisms of apoptosis induced by different virus strains.
Our own German TBEV isolate MucAr AS33 (western subtype) and our Mongolian TBEV isolate MucAr M14/10 (Siberian subtype) we compared with the two reference strains Hypr (highly pathogenic) and Langat virus (low pathogenic) for growth dynamics in different cell lines and plaque formation.
Virus strains were adapted to the cell lines and growth curves were determined. Growth titers were tested in parallel with viral RNA by RTQ-PCR. Plaque sizes of the strains were compared to each other. First results show differences in the titers and the growth curves for each cell line. Virus replication in the neuroblastoma cells was found to be faster with higher virus titers than in glioblastoma cells.

P11

Non-zoonotic and zoonotic MRSA: Factors promoting colonization and defence resistance in humans and animals

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Keywords: LA-MRSA, zoonosis, adhesion, phagocytosis

Since recently livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) are recognized as a source for human infections with a potential for a major healthcare challenge, yet, the pathogenic mechanism of endemic LA-MRSA and of their Methicllin-sensitive counterparts for species barriers transmission, colonization, and disease formation are largely unknown. Crucial steps towards disease include bacterial adhesion to host cell matrix components, invasion of cells and tissues, and evasion from host immune response.
Accordingly, we are planning to address these processes in a multi-level approach: We will analyze the ability of epidemiologically relevant zoonotic MRSA and MSSA to adhere to human host cell matrix components and to defined cells from animals and humans. In a second approach, we will study the uptake of zoonotic and non-zoonotic MRSA and MSSA by professional and non-professional porcine and human blood phagocytes, and the survival of the staphylococci after phagocytosis. At this stage, we looked for potential differences between paired zoonotic MRSA isolated from human and animal (livestock/farm personnel, and pet/pet-owner). First preliminary results indicate a clear strain to strain variation in the pathogens abilities to adhere to immobilized human fibronectin. Similarly, we found clear differences in the fibronectin binding capacities in some of the strain pairs. However, no clear differences in fibronectin binding were identified so far between LA-MRSA obtained from these two sources. Similarly, in whole blood phagocytosis assays, we found clear differences in the bacterial uptake by porcine and human granulocytes, but no such differences in terms of the source (animal or human). Taken together, our data suggest that zoonotic MRSA derived from animals cannot be easily discriminated from those derived from humans in terms of adhesion and immune evasion (phagocytosis).

P12

Staphylococcus aureus-induced cell death and invasiveness of human host cells as virulence mechanisms of zoonotic isolates

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Keywords: *S. aureus*, cellular invasion, cytotoxicity

Staphylococcus aureus lineages have been considered generally as host-specific, existing as different biovars. This may have changed, since some lineages are now apparently crossing host species barriers. *S. aureus* is an important human and animal colonizer and pathogen and has been traditionally regarded as an extracellular pathogen, but is now considered as a facultative intracellular bacterium. However, the precise role of invasiveness and of other properties (e.g. cytotoxicity) are only partially understood.

This project will address whether there are differences between animal-derived and human-derived *S. aureus* strains focussing on two steps of microbial pathogenesis. We will use an integrated approach to type and characterize human and animal-derived isolates for cellular invasiveness and cytotoxicity on cognate and non-cognate cell lines. In addition, transcriptional activity of selected global regulator genes implicated in virulence will be quantified. Taken together this will allow to obtain a more comprehensive view of the *S. aureus* population with regard to the virulence characteristics and to identify the role of host cell invasion and cell death induction for colonization and infection in the *S. aureus* population.

The mechanisms and virulence factors identified here will facilitate a risk assessment with regard to virulence, as well as help to identify potential candidate genes for vaccine development. Identified virulence factors can be used as additional diagnostic tools for implementation of tailored treatment and prevention strategies for management of changing epidemiologic challenges of spread of resistance by newer lineages, such as zoonotic MRSA.

P13

Identification and characterization of *Coxiella burnetii* type IV secretion system effector proteins that prevent apoptosis

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Keywords: apoptosis, *Coxiella burnetii*, Secretion system, effector protein

Apoptosis is an important innate immune response used by mammalian cells to control infection by intracellular pathogens. Hence, bacteria have developed multiple strategies to modulate the host cell death signaling pathways. Some bacteria actively induce cell death in their host cell to remove immune effector cells or to overcome barriers. Alternatively, intracellular pathogens often inhibit host cell death to preserve their replicative niche. *Coxiella burnetii*, an obligate intracellular pathogen that causes Q-fever in humans, has been shown to inhibit the intrinsic and the extrinsic apoptosis pathways. How *Coxiella burnetii* prevents apoptosis in its host cells is not well understood. We showed, that a unique *Coxiella burnetii* type IV secretion system effector protein called AnkG inhibit pathogen-induced host cell apoptosis, possibly by the interaction with the host protein p32 (gC1qR). AnkG inhibits the intrinsic apoptosis pathway, but does not influence the extrinsic apoptosis pathway. However, *Coxiella burnetii* inhibit both apoptosis pathways. Therefore, we screened 20 newly identified *Coxiella burnetii* type IV secretion effector proteins in order to identify proteins that interfere with the extrinsic apoptosis pathway. Here, we present 4 *Coxiella burnetii* effector proteins that are capable to inhibit the induction of the extrinsic apoptosis pathway. These four effector proteins showed different intracellular localization when ectopically expressed, suggesting that they might interact with different cellular partners and thereby inhibit the extrinsic apoptosis pathway at different steps. We are currently investigating the function of these four effector proteins on a molecular level in order to understand their anti-apoptotic activity.

P14

Novel aspects of host-pathogen interaction mediated by immunoglobulin-binding protein EibG from Shiga toxin-producing *Escherichia coli*

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Keywords: STEC, immunoglobulin-binding protein, receptors, peptide mapping

Shiga toxin-producing *Escherichia coli* (STEC) can cause diarrhea and the hemolytic uremic syndrome (HUS) in humans and thus belong to a group of pathogens of major interest. Several related immunoglobulin (Ig)-binding proteins namely EibA, C, D, E, F, G and H are expressed by different STEC strains. These proteins share the facility to bind IgG by nonimmune manner, whereby the potential evasion of host immune response is possible. Within a single strain Eib-proteins can occur separately or combined. Additionally, EibG is involved in bacterial adherence to host intestinal epithelial cells and *eibG*-positive strains show chain-like adherence pattern (CLAP) depending on different *eibG*-allels.
Sequencing of entire *eibG* revealed 21 different *eibG*-allels which were allocated to the three subtypes *eibG*-α, -β and –γ. Different CLAP-phenotypes depending on *eibG*-subtypes were shown in adhesion assays with human and bovine epithelial cells. These results were confirmed by inserting *eibG* into *E. coli* with alternative genetic background. Separation of outer and inner membranes of *eibG*-positive *E. coli*, based on the preparation of spheroplasts and ensuing sucrose density gradient centrifugation, allowed novel insight into multimerisation of EibG. Peptide mapping of EibG variants by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and comparison to sequences of other Eib-proteins should help to better understand the molecular mechanisms of IgG-binding and cellular adherence. In search of cell surface receptors, binding studies of EibG with glycosphingolipids of animal cells by thin-layer chromatography (TLC) overlay assay

techniques will provide further insights into EibG-mediated molecular cell surface interaction.

P15

Identification and characterization of host-specificity determinants of *Salmonella enterica* serovars for swine and man

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Keywords: Salmonella, host-specificity, host immune response, AvrA protein, NFκB activity

This project addresses two aspects of the zoonotic potential of *Salmonella* serovars: The ability of different serovars to infect, survive and proliferate within human and porcine host cells and the ability for the innate immune response to limit the progress of infection. Currently, we are studying the *Salmonella* virulence protein AvrA. AvrA has been reported to modulate host cell innate immune responses by inhibition of the host transcriptional activator NFκB. Interestingly, the *avrA* gene is not present in serovars of *Salmonella* associated with systemic host infections, such *S.* Typhi and *S.* Choleraesuis, which has suggested a model whereby the absence of AvrA correlates with the tendency for certain serovars to cause systemic infections.
In invasion assays *S.* Choleraesuis, showed higher rates of intracellular growth in the porcine macrophage cell line compared to *S.* Typhimurium. This observation is consistent with the hypothesis that the ability to survive and proliferate within porcine macrophage and possibly other immune cells may play a role in both host-adaptation of *S.* Choleraesuis and its higher rates of systemic infections in both humans and swine.
In order to determine the role of AvrA in host adaptation and/or pathogenesis, we cloned the gene from *S.* Typhimurium and introduced it into *S.* Choleraesuis, which does not have the gene. These clones are being use to determine the virulence properties of an AvrA+ Choleraesuis strain as well as determination of the effects on NFκB activity.

P16

Integrated microRNA-mRNA-Analysis of Human Monocyte Derived Macrophages upon Mycobacterium avium subsp. hominissuis Infection

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Many efforts have been made to understand basal mechanisms of mycobacterial infections. Macrophages are the first line of host immune defence to encounter and eradicate mycobacteria. Pathogenic species have evolved different mechanisms to evade host response, e.g. by influencing macrophage apoptotic pathways. However, the underlying molecular regulation is not fully understood. A new layer of eukaryotic regulation of gene expression is constituted by microRNAs. Therefore, we present a comprehensive study for identification of these key regulators and their targets in the context of host macrophage response to mycobacterial infections.
We performed microRNA as well as mRNA expression analysis of human monocyte derived macrophages infected with several *Mycobacterium avium hominissuis* strains by means of microarrays as well as quantitative reverse transcription PCR (qRT-PCR). The data revealed the ability of all strains to inhibit apoptosis by transcriptional regulation of BCL2 family members. Accordingly, at 48 h after infection macrophages infected with all *M. avium* strains showed significantly decreased caspase 3 and 7 activities compared to the controls. Expression of let-7e, miR-29a and miR-886-5p were increased in response to mycobacterial infection at 48 h. The integrated analysis of microRNA and mRNA expression as well as target prediction pointed out regulative networks identifying caspase 3 and 7 as potential targets of let-7e and miR-29a, respectively. Consecutive reporter assays verified the regulation of caspase 3 and 7 by these microRNAs.
We show for the first time that mycobacterial infection of human macrophages causes a specific microRNA response. We furthermore outlined a regulatory network of potential

interactions between microRNAs and mRNAs. This study provides a theoretical concept for unveiling how distinct mycobacteria could manipulate host cell response. In addition, functional relevance was confirmed by uncovering the control of major caspases 3 and 7 by let-7e and miR-29a, respectively.

P17

Invasion and anti-apoptotic activities of *Mycobacterium avium* subsp. *paratuberculosis* in epithelial cells

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Mycobacterium avium subsp. *paratuberculosis* (MAP), the etiologic agent of paratuberculosis in cattle, is suspected to be associated with human Crohn´s disease (CD), a chronic inflammatory bowel disease, though the zoonotic potential of MAP is controversially discussed. A critical step in the pathogenesis of MAP infections is the invasion of intestinal epithelial cells by MAP.
The aim of our study was to analyse the ability of MAP to invade and survive in intestinal bovine epithelial cells (FKD-R) compared to human cells (Caco-2). Both cell lines were inoculated with MAP strain (ATCC 19689) at a MOI of 100 for 4 h. After incubation, extracellular bacteria were killed by amikacin (200 µg ml^{-1}) treatment, supernatants were replaced by fresh medium and cells were further cultured for 3-24 d. Finally, cells were lysed with Triton X-100 (0.1 %) and serial dilutions were plated for colony counts.
MAP was able to invade both epithelial cell lines and persisted intracellularly. The number of viable intracellular bacteria remained constant over the entire observation period. Infected FKD-R cells formed a closed cell monolayer and showed no signs of increased cell death. By contrast, uninfected cells significantly changed cell morphology within 10 days and succumbed to cell death until day 12.
MAP seems to have the ability to inhibit apoptosis in bovine epithelial cells during infection. Further investigations will aim to elucidate the anti-apoptotic mechanisms involved.

P18

Growth characteristics of *Rickettsia helvetica* strain AS 819 in different eukaryotic cell lines

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Keywords: *Rickettsia helvetica,* pathogen-host interaction

Rickettsia (*R.*) *helvetica* is the most prevalent spotted fever group (SFG) *Rickettsia* found in *Ixodes* ticks in Germany. In humans, *R. helvetica* infection has been associated with aneruptive fever, headache, myocarditis and meningitis. Studies regarding the pathogenicity of this agent have not been reported so far. We investigated growth characteristics of *R. helvetica* in order to determine ultra-structural changes within different host cell lines, to look for properties commonly described in other SFG *Rickettsia*, and to propose putative mechanisms of pathogenesis. African green monkey kidney epithelial cells (Vero E6) and mouse fibroblast cells (L929) were infected with low passage wild type *R. helvetica* (strain AS 819) isolated from ticks. At 24 h intervals cells were fixed and prepared for confocal laser scanning microscopy over a period of 21 days. Immunofluorescent staining of cell structures included actin, nucleus, and alpha-tubulin. Growth of *R. helvetica* within Vero E6 and L929 resulted in similar amounts of *R. helvetica*-DNA as determined by quantitative real-time PCR. Detection of DNA was performed on culture supernatants and compared to freeze-thawed cells. The number of copies/µl measured in Vero E6 freeze-thawed cells was up to 1,000x higher compared to the corresponding cell supernatant. This phenomenon was not observed for L929 cells. Confocal laser scanning microscopy revealed differences in the intracellular distribution of *R. helvetica* compared to other SFG members. Intracellular actin polymerisation-based motility was not observed. We assume that the results of our investigations reveal an interesting insight in the pathogenicity of *R. helvetica* leading to a better understanding of the relationship between growth characteristics and clinical manifestation of *R. helvetica*-infection.

P19

New insights on the *T. gondii* antigen GRA9

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Keywords: toxoplasmosis, GRA-proteins

The apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular parasite which can infect nearly all warm blooded animals including humans. In host cells the parasite develops within a parasitophorous vacuole (PV), which causes handicaps in nutrient supply. GRA- and ROP-proteins, the main components of the PV are delivered from secretory organelles and are important in the connection between parasites and host cell organelles. We have described a new *T. gondii* protein called GRA9 which stably associates with the network of membranous tubules and the PV membrane (PVM). We show here that GRA9 co-localize with the dense granules proteins GRA1, GRA2, GRA3, GRA5 and GRA7. Recent co-immunoprecipitation data indicate that GRA9 is a major interaction partner of GRA7 and GRA3 which both were found to be important in nutrient transport between the host cell and the parasite. To explore GRA9 secretion into the PV and the interaction with network structures and the PVM in more detail, we exogenously expressed HA-tagged GRA9 as well as C-terminal truncated versions of GRA9 in RH tachyzoites. Results indicate that the C-terminus of GRA9 is essential and sufficient for the correct GRA9 secretion into the PV. GRA9-proteins lacking the last one hundred amino acids, including two hydrophobic domains show an alteration in secretion behaviour. The C-terminal truncated GRA9-protein was found exclusively within the cytoplasm of the parasites and could not enter the lumen of the PV.

(This project is part of TOXONET, which is being supported by a grant from the BMBF).

P20

Differentiation of the host cell impacts *Toxoplasma gondii* development in skeletal muscle cells

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Keywords: *Toxoplasma gondii*, skeletal muscle cells, *T. gondii*-host cell interaction, differentiation, MAP kinases

Transmission of *Toxoplasma gondii*, a zoonotic apicomplexan, to humans occurs regularly via the intake of bradyzoite-containing undercooked or cured meat products. Parasite development within skeletal muscle cells (SkMCs) may be particularly important for this route of transmission. In this study, we are determining cell-type-specific factors which control parasite development and persistence in SkMCs.
The interaction of *Toxoplasma* with SkMCs was investigated in undifferentiated murine C2C12 myoblasts, differentiated C2C12 myotubes and NIH3T3 control fibroblasts. Quantification of *T. gondii* using PCR indicated that the parasite replicates stronger in undifferentiated myoblasts than in differentiated myotubes. Parasite numbers were higher in parasitophorous vacuoles from myoblasts or NIH3T3, than in those from myotubes. In addition, host cell lysis due to parasite replication was more evident in undifferentiated myoblasts and NIH3T3 than in differentiated myotubes. The MAP kinases p38 and ERK as well as Akt/PKB regulate SkMC differentiation and this was confirmed herein by their increased phosphorylation during C2C12 differentiation. Furthermore, C2C12 myotubes showed increased activation of p38 and Akt/PKB proteins after *T. gondii* infection as compared to C2C12 myoblasts and NIH3T3 suggesting an interaction between MAPK activation, C2C12 differentiation and *T. gondii* development. These results indicate that the host cell type and the SkMC differentiation impact *T. gondii* replication *in vitro*. This might also explain the different efficiencies with which *T. gondii* persists in various host tissues.

Poster Session – Epidemiology, surveillance and risk assessment

E01

Pilot project presentation: Foundations of a functional epidemiology of *Giardia duodenalis* infections

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This pilot project aims at initiating a *Giardia duodenalis* biobank and database holding environmental and clinical parasite isolates from human and veterinary cases that links organisms with relevant epidemiological and/or clinical information.
An update will be presented on the streamlined workflow that we developed to generate the biobank and on the associated database-application that collates epidemiological and biological data, e.g. genotyping results, culture conditions, and stockpiling of *Giardia* spp isolates.
Furthermore, we will introduce assays to classify isolates functionally that we developed or adapted specifically for the project and present first results.

E02

Fundamental investigations for functional epidemiology of *Giardia duodenalis* infections

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Keywords: *Giardia duodenalis*, biobank, arginine deiminase, dendritic cells

The protozoa *Giardia duodenalis* (*Gd*) is an intestinal parasite of humans. It causes diarrhoeal and gastrointestinal disease, named giardiasis. Annually, around 300 million people become infected worldwide. *Gd* is ubiquitously distributed and also infects other vertebrates, including companion animals. Thus, giardiasis is a major public and veterinary health concern globally. It remains unclear to what degree it is a zoonosis and what determines its pathogenicity to humans. To assess these issues a *Gd* biobank shall be generated to link epidemiological data with functional characteristics of clinical isolates and as a means to identify virulence associated genes. We hypothesize that potential virulence markers are enzymes released by *Giardia* upon colonization of the small instestine and interaction with the host epithelium. Especially the *Gd* arginine deiminase (ADI) that converts arginine to citrulline and ammonia shall be investigated as possible virulence and pathogenicity factor of *Gd*, because arginine plays a critical role in antimicrobial mechanisms and immune response. The sequence-variation of ADI will be determined by cloning *adi* from *Gd* isolates being collected in the biobank and sequence variants will be functionally characterized by expressing the respective recombinant ADI proteins. Finally, correlation between enzyme function and clinical pathology shall be investigated on basis of *Gd* isolates related epidemiological data.
We will present the establishment of this workflow. *Gd* clone WB 6 ADI as well as a mutated ADI (C424A) were cloned,

recombinantly expressed and purified by affinity chromatography. An enzyme activity test was adapted for colorimetric measurement of arginine to citrulline conversion to verify active and inactive ADI. Subsequently, *in vitro* generated human dendritic cells (DCs) were treated with recombinant ADI or *mut*ADI (C424A) in absence and presence of Toll-like receptor (TLR) ligands. Investigations of surface molecule expression and cytokine release shall give first evidences for ADI and its role for host-parasite interplay.

E03

Experiences with Coxevac® in sheep

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Keywords: Q fever, C. burnetii, Coxevac®, vaccination, sheep

After a Q fever outbreak associated with human infections at an experimental station on the Swabian Alb in 2009 the sheep flock was regularly examined for *Coxiella (C.) burnetii* and a sanitation programme implemented. Besides hygiene measures the flock was vaccinated with Coxevac®, which is licensed for the European Market for goat and cattle since September 2010. A vaccination study including 40 ewes and gimmers was carried out at this farm.

Before vaccination all individuals were tested serologically negative for Q fever and no shedding of *C. burnetii* was observed. Around the initial immunisation rectal temperature, general condition and the local reaction were controlled. Serum samples were checked for antibodies against *C. burnetii*. EDTA blood, vaginal and faecal swabs were proved for *C. burnetii* per PCR until 20 weeks after the first shot.

All individuals showed a good general condition. A slight rise in temperature was measured within the first days after vaccination. During the whole investigation no shedding of *C. burnetii* was detected. In a small group of animals a minor swelling at the injection site was observed. Positive antibody activity was detectable in 7,5% of all animals at the date of the second shot, in 52,5% 3,5 weeks after initial immunisation, and in 37,5% another 5 weeks later.

In this trial only 52,5% of the sheep were seropositive 3,5 weeks after the second shot contrary to flocks with acute Q fever, where 98% of all animals were seropositive at this time after vaccination.

E05

Q fever: Baseline monitoring of a sheep and a goat flock associated with human infections

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Keywords: Q fever, *Coxiella burnetii*, sheep, goats, shedding routes

Animal losses due to abortion and weak offspring during a lambing period amounted up to 25% in a goat flock and up to 18% in a sheep flock. Both flocks were kept at an experimental station on the Swabian Alb, Germany. Fifteen of 23 employees and residents on the farm were tested positive for *Coxiella (C.) burnetii* antibodies by ELISA and IIFT. The animals were investigated using an ELISA. The different shedding routes and EDTA blood samples of the animals were analyzed by PCR. In goats 94% and 74% of the sheep were respectively seropositive for *C. burnetii*. Blood samples of 8% goats and 3% sheep were also positive. *C. burnetii* was shed by vaginal mucus (100%), by milk (97% in goats and 78% in sheep) and by faeces (100% only tested in sheep).
In this case study the coherence between human and animal infections was obvious.

E06

Investigation on the prevalence of *Coxiella burnetii* in clinically healthy sheep flocks

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Keywords: The German Q fever network, *Coxiella burnetii*

Coxiella (C.) burnetii causes Q fever, a highly infectious disease affecting humans as well as livestock. The primary reservoirs of the agent are goats, cattle, and sheep. Current epidemiological data on the situation of *Coxiella burnetii* infections in sheep are missing, making risk assessment and the implementation of counteractive measures difficult.
Using the German state of Thuringia as a model example, the sero-, and antigen prevalence of *C. burnetii* (estimated at 25 %) was assessed in forty randomly selected clinically healthy sheep flocks. Serum samples, vaginal and rectal swabs, as well as afterbirths, and foetuses/foetal swabs were collected and analysed.
Our investigations revealed that *C. burnetii* is present in Thuringian sheep flocks and sporadic flare-ups do occur. Although *C. burnetii* infections are not primarily a veterinary concern due to the lack of significant impact on animal health, the zoonotic risk for humans should not be underestimated. Therefore, strategies combining the interests of public and veterinary public health should include monitoring of flocks, the identification and culling of shedders as well as the administration of protective vaccines.

E07

Infection of humans with *Salmonella enterica* serovar Paratyphi B (dT+) is mainly caused by reptiles and rarely by poultry

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Keywords: Salmonella, Paratyphi B (dT+), infection, molecular typing

Salmonella enterica serovar Paratyphi B (dT+), formerly called Java, has been reported in humans with sporadic cases and outbreaks. We have investigated the potential sources of human infection due to this serovar by comparative typing of isolates from animals, food, the environment and humans received at the NRL-Salm and NRZ-RKI over the last ten years. The majority of strains isolated from livestock (mainly poultry), food and the environment did not express the O:5-antigen. Strains isolated mainly from reptiles expressed the O:5-antigen. In contrast, strains from humans expressed almost always the O:5-antigen (O:5+) with only a few exceptions. A selection of altogether 113 epidemiologically independent strains were further typed by MLST and PFGE to clarify whether Paratyphi B (dT+) O:5+ strains from humans and reptiles belong to one clonal group. Results clearly indicate that the human and reptile isolates tested share mainly the sequence type ST88. Antigen O:5- strains from poultry (including products thereof) share a distinct sequence type ST28 which differed from ST88 in all 7 loci. PFGE patterns within ST28 were identical or highly similar. Antigen O:5- strains from humans are rather heterogeneous in their STs. We conclude that the infection of humans with *Salmonella enterica* serovar Paratyphi B (dT+) is mainly caused by contact with reptiles and scarcely by meat from poultry or pork. Strains with ST28 seem to cause rarely an infection in humans but are continuously and frequently isolated from broilers suggesting that this type is rather adapted to poultry.

E08

Poultry breeders exposure to Salmonella Typhimurium in chicken farms

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The prevalence of Salmonella Typhimurium in chicken farms located in Menufiya province, Egypt, and their poultry breeders exposure were investigated in current study as a zoonotic problem. Cloacal swabs (n=200), litter samples (n=200) and 27 poultry breeder stool samples were collected from 8 chicken farms. All samples were examined for Salmonella Typhimurium occurrence using DIASALM medium and Rambach agar. Serotyping was confirmed with Kaufmann-white scheme. Exposed farms recorded 10% and 13% prevalence of Salmonella Typhimurium in cloacal swabs and litter samples respectively, whereas non-exposed farms pointed out 2% for each of them. The poultry breeder stool samples demonstrated 53.8 and 7.1 percentages in exposed and non-exposed farms respectively, while the relative risk between them was 7.54 (95% CI, 1.07 to 53.23) at P = 0.043. There was a significant difference and increased risk for poultry breeders in exposed farms comparing with non-exposed one. In conclusion, using of antibiotics as a preventive measure for chicken Salmonellosis is recommended for poultry breeders' protection.

E09

Source attribution – a German approach for Samonella spp.

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Keywords: Source attribution, microbial subtyping, model, Salmonella

Zoonoses have a major impact to human health. Salmonellosis is one of the most common and widely distributed foodborne diseases. According to the RKI, in 2009 about 31.000 people have been infected in Germany. Therefore, the reduction of Salmonella at all stages from 'farm to fork' still is of great importance.
Among experts there is an ongoing discussion about the contribution of animal related Salmonella agents to human infections. In 2004 Tine Hald presented a strong tool to examine those relations, a source attribution method based on a microbial subtyping approach.
In this mathematical model, the number of sporadic cases of human salmonellosis caused by different sero- and phage types was calculated as a function of these types in the animal-food sources and the amount of each food item consumed. It delivers the estimated contribution of each food source to human infections with Salmonella.
The EFSA strongly recommends this widely established method to explore new intervention points to reduce Salmonella in the food chain and to proof the reliability of already operating strategies.

The poster shall give a short insight into the method of source attribution and our first results on the German samonellosis situation.

E10

Sequence analysis and phylogeny of the multi-resistance plasmid pSTE1 from *Staphylococcus hyicus*

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Keywords: horizontal gene transfer, recombination, integration

Background: The aim of this study was to determine the complete sequence of the first staphylococcal *tet*(L)-carrying multi-resistance plasmid pSTE1 and to determine its phylogeny.
Methods: The *Staphylococcus hyicus* plasmid pSTE1 was isolated from a piglet suffering from exudative epidermitis in the late 1980s. The plasmid was transformed into *S. aureus* RN4220. BglII/BclI, EcoRI or HindIII fragments were cloned, sequenced, compiled and analysed.
Results: Plasmid pSTE1 is 11,957 bp and carries – besides the tetracycline resistance gene *tet*(L) – another two resistance genes: the streptomycin resistance gene *aad(6)* and the macrolide-lincosamide-streptogramin B resistance gene *erm*(B). The initial 3 kb of pSTE1 corresponded closely to plasmid pK214 from *Lactococcus lactis* and included a plasmid replication gene *rep*, the *aad(6)* gene and the 5′ end of a mobilization gene (*mob*). Further downstream, the left terminus including the *erm*(B) gene of the *Enterococcus faecalis* transposon Tn*917* was detected. A 0.6 kb segment of the *Lactobacillus reuteri* plasmid pLR585 connects this part with a 2.3 kb region of the *Bacillus cereus* plasmid pBC16 which contains the *tet*(L) gene. The remaining parts of plasmid pSTE1 comprise segments which are homologous to the staphylococcal plasmids pSCFS1 and pKKS825, and contain *pre*/*mob* and *rep* genes.
Conclusions: Plasmid pSTE1 seems to have developed from interplasmid recombination events and the integration and truncation of a small non-conjugative transposon. The three resistance genes of plasmid pSTE1 originate from three different Gram-positive genera: *Lactococcus*, *Enterococcus* and *Bacillus*. This finding underlines the role of plasmids in the dissemination of resistance genes among Gram-positive bacteria.

E11

Molecular characterization of MRSA from cattle and poultry detected in the GE*RM*-Vet program 2008 and 2009

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Keywords: MRSA, cattle, poultry, typing, antimicrobial resistance

Objectives: The aim of this study was to characterize MRSA isolates from cattle and poultry obtained during 2008 and 2009 in the German national veterinary resistance monitoring program GE*RM*-Vet.
Material and Methods: In total, 11 (1.5%) of 720 bovine and 2 (2.1%) of 96 avian *Staphylococcus aureus* isolates of the GE*RM*-Vet studies 2008 and 2009 were methicillin-resistant. These isolates were investigated for their *spa*, SCC*mec*, and *dru* types and by using two CC398-specific PCRs. For non-CC398 isolates, MLST was performed. Broth microdilution was conducted for 29 antimicrobial agents and the respective resistance genes were detected by PCR.
Results: Eleven isolates belonged to CC398 and single isolates to ST5 and ST9, respectively. The *spa* type t011 was found in nine isolates, while *spa* types t002, t034 and t1430 were present in one or two isolates. Eleven isolates belonged to SCC*mec* type V and single isolates to types III and IV. Four *dru* types were identified with dt11a being present in ten isolates and dt6r (a novel type), dt9v and dt10a in single isolates. Susceptibility testing revealed six resistance patterns with all isolates being resistant against β-lactams and tetracyclines. The respective resistance genes *mecA*, *blaZ* (β-lactams), *tet*(K/L/M) [tetracyclines], *erm*(A/B) [macrolides/lincosamides/streptogramin B], *dfrK* [trimethoprim], *aacA-aphD* [gentamicin/kanamycin] and *spc* [spectinomycin] were detected.
Conclusion: MRSA isolates were found rarely in diseased cattle and poultry in the GE*RM*-Vet studies. The occurrence of ST5 (poultry) and ST9 (cattle) isolates may point towards an exchange of MRSA with humans and other animal species.

E12

Genetic comparison of livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) against a temporal and spatial background

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Keywords: Livestock-associated MRSA, genetic diversity, MLVA

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen mostly associated with hospital- and community-acquired infections. In recent years, MRSA have also been found to colonize and infect livestock and studies have indicated that MRSA can be transmitted from animals to humans and vice versa. Livestock-associated MRSA is therefore regarded as an important zoonotic agent. To unravel the transmission dynamics and genetic diversity of zoonotic *S. aureus* strains, precise typing methods with a high epidemiological resolution are of great importance. However, MRSA-isolates from livestock are mainly associated with a limited subset of *S. aureus* protein A (*spa)* types, most of which belong to the clonal complex 398 according to multilocus sequence typing (MLST).
In this study, we apply multiple-locus variable number of tandem repeats analysis (MLVA) which has recently been described as an alternative and highly discriminative tool for *S. aureus* typing to investigate the genetic background of zoonotic MRSA isolates derived between 2005 and 2010 from more than 50 pig stables of various locations in Germany.
First typing results show that strains of different geographic regions that are indistinguishable by *spa*-typing can be clustered into at least two different MLVA-types. Yet, the MLVA profiles identified so far differ in only one locus which supports the theory of a clonal origin of livestock-associated MRSA.
Our findings might contribute to a better understanding of the transmission dynamics and the genetic diversity against a temporal and spatial background of a pathogen that has only recently overcome a species barrier.

E13

MRSA in the environment of pig farms

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Keywords: MRSA, multiresistant bacteria, aerosol, animal farming, epidemiology

So called livestock associated MRSA (methicillin resistant *Staphylococcus aureus*) occurs in different farm animals. Livestock associated MRSA predominantly belongs to a special sequence type of MRSA which usually not appears in humans but a transmission from animals to humans is for sure. However, the knowledge on occurrence and transmission of MRSA in livestock are still scarce.
Therefore studies funded by the Federal Ministry of Food, Agriculture and Consumer Protection about the occurrence of MRSA in stable air and exhaust air as well as in other environmental samples in pig stables were made.
27 pig barns of different sizes and management schemes were investigated. Samples of animals (nasal and skin swabs) as well as samples of the environment such as air samples (impingement and filtration), boot socks, pooled samples from dust, faeces and feed were collected.
In barns chosen for the long-term-studies air samples of the exhaust air were taken.
There is a high occurrence of MRSA in animal samples as well as in all environmental samples including air samples inside the stable.
MRSA could be detected in exhaust air samples on the downwind side in three out of six investigated barns. It seems that MRSA are emitted from the analysed barns by way of the air. Further investigations are necessary to confirm the results.

E14

Enrofloxacin resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) of poultry origin

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Keywords: fluoroquinolones, DNA gyrase, DNA topoisomerase IV

Background: Enrofloxacin is a fluoroquinolone (FQ) routinely used to treat infections in food-producing, pet and companion animals. Mechanisms involved in FQ resistance in MRSA are amino acid alterations in the target enzymes of quinolone agents (GyrA/B and GrlA/B), or overexpression of the *norA* gene encoding a multidrug efflux pump.
Methods: A total of 32 MRSA isolates of poultry origin were tested for enrofloxacin MICs by broth microdilution. The prevalence of mutations in the genes *gyrA, gyrB*, *grlA* and *grlB*, and in the promoter region of the *norA* gene were analysed by sequencing of PCR products.
Results: Five isolates were classified as resistant and four as intermediate by their MICs of 8 – ≥32 mg/L, and 1 – 2 mg/L, respectively. All nine isolates exhibited mutations which led to the amino acid exchanges Ser80Phe in GrlA, but the alteration Ser84Leu in GyrA was only detected in resistant isolates. In addition, one isolate revealed a mutation in *gyrB* which resulted in the exchange Arg517Lys while another five isolates had a mutation in *grlB* which led to the exchange Glu422Asp. Moreover, the isolates showed some point mutations in the *norA* promoter region, which, however, did neither affect the -35 and -10 positions, nor the *norA*-associated ribosome binding site.
Conclusions: Enrofloxacin resistance of MRSA isolates of poultry origin is mediated by *gyrA* and/or *grlA* mutations, which were identical to those reported previously in MRSA isolates of different origin. The newly described *gyrB* and *grlB* mutations did not additionally increase the MIC of enrofloxacin.

E15

Differences between patients colonized with livestock- vs. healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA)

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Keywords: LA-MRSA, livestock, zoonosis, CC398, DRG

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) can be transmitted to humans exposed to these animals. Moreover, such strains can be introduced into healthcare facilities. Until now, it is not well understood how LA-MRSA, which are mostly characterized by the clonal complex (CC) 398, spread in hospitals. Therefore, the purpose of this case-control study is to assess, whether structural differences between patients colonized with LA-MRSA and patients carrying healthcare-associated MRSA (HA-MRSA) molecular clones contribute to divergent transmission or infection rates.

All MRSA isolates from inpatients admitted in 2008 and 2009 to a university-hospital were characterized by *S. aureus* protein A gene (*spa*) typing. Data of patients colonized with LA-MRSA were compared to patients carrying HA-MRSA with respect to age, sex, length of stay (LOS) in the hospital and on intensive care units (ICUs), as well as medical procedures and diagnosis-related groups (DRGs) coded for the two groups of patients.

We found that LA-MRSA patients were significantly younger, had a shorter average hospital LOS and were less frequently admitted to ICUs. Further significant differences between LA- and HA-MRSA patients were observed for the mean numbers and types of diagnoses and medical procedures performed.

Overall, patients colonized with LA-MRSA differed from those colonized with HA-MRSA isolates regarding several characteristics, which could help to explain differences in the likeliness for

healthcare-associated transmission and the incidence of infections caused by different MRSA lineages.

E16

Insights into genetic variability of methicillin resistant *Staphylococcus aureus* (MRSA) from companion animals in Germany

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Keywords: MRSA, companion animals

Methicillin resistant *Staphylococcus aureus* (MRSA) are one of the major causes of wound infections not only in humans, but also in small animals and horses. In addition, colonization with livestock-associated (LA-) MRSA seems to be widely distributed among pigs and cattle.
To gain general and basic knowledge about the proportion and frequency of MRSA- genotypes associated with wound infections in small animals and horses, 1.814 wound swabs were investigated between Nov 2010 and May 2011 for the presence of MRSA. The isolated MRSA were further characterized by macrorestriction analysis (PFGE) *spa*-typing and multi locus sequence typing (MLST). The distribution of MRSA among samples obtained was as follows: dogs (4.9%), cats (5.8%) and horses (9.1%). Within MRSA of canine (n = 59) and feline origin (n = 23), 12 / 10 different *spa* types were detected, respectively. Most of these *spa*- types were known to be associated with clonal complexes (CC) 5, 8, and 22. These CCs had been previously reported for both, MRSA of small animal and human origin. In addition, *spa* types t011 and t034, which were frequently associated with LA- MRSA (CC398), were detected in canine isolates. Among equine isolates (n = 20), four different *spa* types associated with the CCs 8 and 398 were found.
These findings clearly corroborate the epidemiological need of an interdisciplinary and collaborative approach to define inter-species transmission of MRSA, as has been now initiated within the BMBF-funded research network MedVet-Staph.

E17

Long-term monitoring of ESBL-producing and Fluoroquinolone resistant Enterobacteriaceae in animal farms and farm environment

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Keywords: ESBL, antibiotic resistances, Enterobacteriaceae, Fluoroquinolones, animal farming

Resistances of Enterobacteriaceae to ß-lactams by the production of extended-spectrum-beta-lactamases (ESBL) and to Fluoroquinolones represent emerging and increasing resistance properties limiting dramatically the therapeutic options in both veterinary and human medicine. Moreover, the presence of ESBL-genes in bacteria of healthy food-producing animals may pose a human hazard. 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 a part of RESET the overall goal of our subproject is the long-term investigation of the prevalence of ESBL-producing and Fluoroquinolone resistant Enterobacteriaceae in pig and poultry farms and their vicinity.
Samples tested were taken from faeces of individual animals as well as the stable environments' interior (pooled faeces, dust, air, boot swabs, litter, flies, mice faeces) and exterior (air, boot swabs, slurry) with regard to different desinfection/antibiotic regimes and housing conditions. Of the seven pig and seven broiler farms under investigation we visited two of each to date. Sampling took place at three different stages during the fattening period.
In the majority of faecal and internal environmental samples ESBL-positive and Fluoroquinolone resistant Enterobacteriaceae

were detected. These microorganisms were also isolated from the stables' external surroundings.
In summary, it seems that ESBL-producing and Fluoroquinolone resistant Enterobacteriaceae have a high occurrence amongst pigs and poultry. The detection of these microorganisms found outside may have originated from within the barn. However, further investigations of other farms and comparing genetically studies are necessary to prove this hypothesis.

E18

Prevalence and antimicrobial susceptibility of pathogenic *Campylobacter coli* recovered from the surfaces of pig livers

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Keywords: Campylobacter spp., liver, pig, antimicrobial susceptibility

Campylobacteriosis is the most prevalent zoonosis in the European Union. Since *Campylobacter* (*C.*) spp. are transmitted primarily through food of animal origin, the presence of antimicrobial drug-resistant *Campylobacter* in raw meat has important public health implications. *C. coli* is a common commensal bacteria of the intestinal tract of pigs, although the agent is rarely detectable on carcasses after overnight chilling. But edible offal might also be the source of transmission into the food chain.

The purpose of the study was to evaluate the prevalence and the resistance pattern of thermophilic *Campylobacter* spp. isolated from liver surfaces of slaughtered pigs.

In a slaughterhouse in Lower Saxony, 1,500 surfaces of pig livers from 50 fattening herds were swabbed during slaughtering process in order to isolate and identify *Campylobacter* spp..

Out of 1,500 swabs, 147 (9.8 %) were positive for *Campylobacter* spp., with *C. coli* as the predominant species (78.9 %), followed by *C. jejuni* (18.4%). Four isolates could not be differentiated. Susceptible testing of 24 *C. coli* isolates from liver surfaces was performed by broth microdilution method and the minimum inhibitory concentrations were determined form 8 antimicrobials resp. antimicrobial combinations: ampicillin, ampicillin/sulbactam, ciprofloxacin, nalidixic acid, genatmicin, tetracycline, erythromycin, trimethoprim/sulfamethoxazole.

The highest resistant rate of *C. coli* isolates was recognized for trimethoprim/sulphamethoxazole (41.7%) followed by

ciprofloxacin (33.3%), tetracycline (29.2%) and erythromycin (20.8%). Resistance to fluoroquinolones and macrolide in *Campylobacter* spp. are interesting from the public health perspective, because both are the drugs of choice for complicated *Campylobacter* infections in humans.

E19

Differentiation of German *Mycobacterium avium* subsp. *paratuberculosis* isolates for epidemiological and phylogenetic investigations

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Keywords: multi-target genotyping, epidemiology, phylogeny
keyword

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiologic agent of paratuberculosis. Having a world-wide distribution nowadays, Germany was one presumptive point of origin of this disease. We aimed to identify MAP genotypes within cattle herds and other hosts in Germany, and to determine their phylogenetic relation to isolates from other regions of the world.
Thirty, epidemiologically unrelated MAP isolates, for which combined IS*900*-RFLP-, MIRU-VNTR- and MLSSR-typing unveiled a high genetic heterogeneity, were selected for Multilocus sequence analysis (MLSA). Detected by specific PCR and RFLP, two of these isolates (sheep isolates) belonged to the MAP-Type-III while the others belonged to the MAP-Type-II group. MAP-Type-II isolates consisted of bovine, red deer, and human isolates including two reference strains (DSM 44135 and ATCC 19698). MLSA showed four sequence types (ST): ST 30 and 32 for MAP-Type-II isolates and ST 35 and 36 for MAP-Type-III isolates. These results correspond to four out of seven sequence types published for MAP isolates from United States, Canada, New Zealand, United Kingdom, Australia, Farao Islands and Iceland. Within the MAP-Type-II group genotypes detected for epidemiological interpretations (combined IS*900*-RFLP-, MIRU-VNTR-, MLSSR-genotypes) did not correspond to those for phylogenetic studies (MLSA).

MLSA-derived phylogeny shows that German MAP isolates are identical to isolates from above-named regions of the world. The fact that four out of seven known MAP ST can already be detected in a limited number of German strains supports the idea that MAP strains distributed all over the world have the same region of origin.

E20

Occurrence of zoonotic pathogens in fattening pig farms

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Keywords: Salmonella enterica, Yersinia enterocolitica, Campylobacter spp.

Campylobacter spp., *Y. enterocolitica* and *Salmonella* spp. represent the three most frequent reported bacterial causes of food associated human enteritis cases. The goal of the study was to assess the transmission pathways and risk factors for these pathogens in pork production.
Thus, the occurrence of the three pathogens was investigated in a comparative cross-sectional study including 50 farms in Lower-Saxony, 25 farms in Salmonella category I, 25 in category III. Culturing of faecal pooling samples, environmental and rodents' samples were conducted. Farm characteristics were evaluated by a questionnaire and by personal inspection.
C. spp. were detected in 33% of all samples taken from the direct environment of the pigs (pen walls, nipple drinkers etc.), almost as much as from faeces. From 8% of these samples *Y. enterocolitica* were isolated. Even at locations without or with only sporadic animal contact (indirect environment) both pathogens were detected: C. spp. from 10% of farmer's boots, *Y. enterocolitica* on 4% of guide boards and passage ways.
No association between the pathogens' occurrence was determined regarding cultural investigation. But *Campylobacter* spp. as well as *Y. enterocolitica* were isolated more often in farms in *Salmonella* category I than category III (*C.* spp. 88% vs. 72%, *Y. enterocolitica* 76% vs. 20%).
It could be shown that *Campylobacter (C.)* spp. and *Y. enterocolitica* occur quite frequently in the animals' environment. Possibly, the impact of the environment regarding the pathogen transmission was underestimated. But it remains unclear, if an entire control strategy against all three pathogens will be possible.

E21

Feasibility study sampling of animals as contribution to veterinary public health in the National Cohort, Germany

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Keywords: feasibility, companion animals, pet owners, swabs

A prospective population-based "National Cohort Study" (NCS) with 200.000 human subjects is currently being planned in Germany. It is primarily designed to address research questions dealing with major chronic diseases. Furthermore, infectious diseases will be examined in a part of the study population.
Since more than 25% of German households own pets, infectious diseases of animals pose a risk to human health. Within a large scale cohort study like the NCS, it is not possible to include examinations of pets of the participants in line with human examinations. Therefore, this feasibility study will test if pet owners can take samples of their pets. For this purpose, both the veterinarian and the pet owner will take swab samples of the nose and mouth of their cat or dog. Swabs taken from the same animal can then be compared with each other. It is also examined if the samples taken by the pet owner are forwarded to the laboratory in a way that allows for microbiological analyses. To gain further information on the status of infection of the animal, its owner is asked for his/her consent for a blood sample of the animal, which is then taken by the veterinarian.
The population of the feasibility study consists of pet owners living in the region of Hanover. 100 pet owners are being recruited, at the clinic for small animals of the University of Veterinary Medicine Hanover, and in a veterinary practice. Sampling is planned to be finished in September. First results will be presented.

E22

Antibody prevalence against 2009 pandemic influenza A (H1N1) virus in Germany: geographically variable immunity in winter 2010/2011

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Keywords: antibody, prevalence, pandemic, influenza A (H1N1), Germany

The prevalence of antibodies against 2009 pandemic influenza A (H1N1) virus was determined in sera of blood donors collected during Winter 2010/11 from several German cities (Hamburg, Stuttgart, Frankfurt, Düsseldorf and Jena) using hemagglutination inhibition assay (HI). For Hamburg, pre-pandemic sera from January 2005 were also tested. A substantial rise in prevalence of antibodies at protective titres (HI≥40), from 47.02% to 86.75%, was observed for Hamburg in Winter 2010/11 compared to the pre-pandemic baseline. However, protective antibody prevalence varied significantly between cities (24.13% to 86.75%) during Winter 2010/2011. This study shows a highly variable immunity among the German population in different major cities shortly after the declaration of the end of the pandemic in August 2010.

E23

GIS Analysis of endemic areas of Borna disease

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Keywords: Borna disease, GIS analysis, endemic areas, horse

Borna disease (BD) is an endemic, sporadically occurring, usually fatal disorder caused by the neurotropic Borna disease virus (BDV). Main natural hosts are horses and sheep but other *Equidae*, farm animals, zoo or companion animals occasionally are diagnosed with natural BD. Clinically manifested equine BD occurs in different endemic areas of southern Germany, Switzerland, Liechtenstein and Austria. A seasonal accumulation of cases in spring and early summer, secular dynamics and the recent detection of BDV infection in shrews in Swiss endemic areas argues for a natural reservoir. Humans can exhibit BDV-specific serum antibodies. Due to these facts, BD represents a suitable model to study the geographical and ecological distribution strategies of rare potential zoonotic agents. Therefore, we established a GIS analysis of the distribution of 450 equine BD cases in Bavarian endemic areas employing data from 1990-2008. The modeling is conducted with the GIS-tool GEPARD 2.0 and is based on a logistic regression. To identify potential reservoirs for BD an additional field study will take place in Bavaria. Small mammals will be caught in endemic and reference areas and tested for the presence of BDV infection. The results of the fieldwork will be incorporated in the model as supplementary data and thus the prediction of BD occurrence will be more precise. This helps us to assess characteristic geographical, climatic and ecological features of endemic areas of BD and to model the potential prevalence and risk for Bavaria but also for other endemic areas or other rare reservoir-bound infectious agents.

E24

Immunological memory to Orthopoxviruses in Germany

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Keywords: risk assessment, cowpox virus, seroprevalence, ELISA

Recently rising numbers of zoonotic cowpox virus (CPXV) infections in humans could be observed in Germany. A possible explanation could be vanishing cross-protection after cessation of the immunisation campaign for the eradication of smallpox with vaccinia virus (VACV). The aim of our study was therefore to evaluate the remaining antibody immunity against orthopoxviruses to give an estimate about the proportion of people potentially unprotected against CPXV infections.
We tested a panel of 800 blood donors from Berlin by ELISA for IgG reactivity against lysed VACV infected cells. A representative subpanel (n = 80) was tested against recombinant OPV proteins produced in *E.Coli* or *Bacculovirus* (A27L, L1R, B5R, A33R, F9L, F13L, H3L, D8L, ATI, A5L). Results were correlated with either age or each other by non parametric spearman correlation. Based on the age distribution in the German population in 2009 an estimate of the remaining antibody immunity was calculated.
We found medium correlations between age and ELISA results (e.g. A27L r = 0.45; VACV r = 0.5972) and high correlations for recombinant proteins and VACV lysate (A33R r = 0.858; B5R r = 0.775). As expected, seropositivity was low in the younger, unvaccinated population (3.1% for < 30 years) and high in the older, vaccinated population (67.3% for 50-59 years). By interpolating our results to the German population we found an overall seropositivity of 37.6%.
Overall our results suggest a raised susceptibility to zoonotic CPXV infections as the majority of the population seems to be unprotected.

E25

Passive surveillance for bat lyssaviruses in indigenous bat species in Germany (1998-2010)

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Keywords: passive surveillance, European bat lyssaviruses

In Germany, rabies in bats is a notifiable zoonotic disease, which is caused by the European bat lyssaviruses (EBLV 1 and 2) and the recently discovered putative new lyssavirus species Bokeloh bat lyssavirus (BBLV). The understanding of bat rabies in indigenous insectivorous bat species is limited. Passive surveillance, i.e. the investigation of dead bats for rabies, is the recommended approach to study the epidemiology of lyssavirus infections in bats. Starting in 1998 a total number of 3021 individuals were tested up to now. Rabies diagnosis was performed using the fluorescent antibody test (FAT) in bat brain samples. Of the 23 indigenous bat species 20 species were represented in this study, originating from 13 of the 16 federal states. The noctule bat (*Nyctalus noctula*) and the common pipistrelle (*Pipistrellus pipistrellus*) were the bat species with the most specimens. Of all investigated bats, 1.2% tested positive for rabies. The vast majority was identified as EBLV-1, predominately associated with the serotine bat (*Eptesicus serotinus*). However, single rabies cases in other species, i.e. noctule bat (*Nyctalus noctula*), Nathusius´s pipistrelle (*Pipistrellus nathusii*), and common pipistrelle (*Pipistrellus pipistrellus*) were also characterized as EBLV-1. In contrast EBLV-2 was only isolated from a single Daubenton's bat (*Myotis daubentonii*).

E26

In vivo survey of *Vibrio* spp. in artificially contaminated *Mytilus edulis*

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Keywords: *Vibrio cholerae*, *Mytilus edulis*, contamination, survival, quantification

All studies performed on the appearance of *Vibrio* spp. in mussels on pre-harvest and retail level in Germany so far showed high loads with these microorganisms. As far as food safety is concerned *Vibrio* (*V.*) *parahaemolyticus*, *V. vulnificus* and *V. cholerae* are the most important species within this genus.
In our study, 106 retail mussel samples of different species and origin were investigated for the presence of *Vibrio*. *Vibrio* spp. was found in 54.8 % of the samples investigated. In approx. 59 % of *Vibrio*-positive samples *V. alginolyticus* was detected, in 12 % *V. cholerae* (non 01, non 0139) and in 6 % *V. parahaemolyticus*. In addition, single *V. metschnikovii, Photobacterium damselae* and *Listonella anguillarum* were found.
One of the most frequently consumed mussel species in Germany and world wide is *Mytilus edulis*. This mussel species filtrates approx. 1.5 l of water per hour, thus an effective clarification occurs in its surrounding. Along with the water, microorganisms such as *V. parahaemolyticus* and *V. cholerae* are affiliated. This potential accumulation of *Vibrio* spp. could cause food borne infections if mussels are not properly heated before consumption. The aim of the study was to determine the clearance rate of *Mytilus edulis* after artificial contamination with *V. cholerae* or *V. parahaemolyticus*. Additionally, the change of *Vibrio* loads in mussels during storage on ice (3°C) or at 15°C were investigated, to simulate post-harvest conditions. Quantitative and semi-quantitative methods were used to describe *Vibrio* concentrations within contamination- and clearance-ponds as well as mussel lumen water, digestive gland and the remaining mussel meat. The contamination assays in *Mytilus edulis* showed in a seven days period an enrichment of *Vibrio* within digestive glands. During clearance a slow but continuously reduction of *Vibrio*

concentrations was found. Within 10 days of storage on ice or at 15°C almost no reduction of the *Vibrio* load occurred in *Mytilus edulis* except for variations in a 2 log interval.

E27

Human hydatidosis in Sudan: Is it a predominant neglected zoonosis or an unseen re-emerging disease?

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Keywords: human, hydatidosis, Sudan, prevalence, strains

Cystic Echinococcosis (CE) is a zoonotic disease affecting mainly various species of livestock and humans. It is caused by metacestodes of dog tapeworms of the Echinococcus granulosus complex. Preliminary data about the prevalence of hydatidosis in different intermediate hosts in Sudan revealed that the disease occurs in all domesticated intermediate host species (camel, cattle, sheep and goats) with the highest infection rates in camels. Nevertheless, infection rates in humans are not very much clear. Moreover, new epidemiological data suggest a major influence of the locally prevailing parasite genotype/species. In this study, a previously described PCR system for species discrimination was used and the partial cox1 and nad1 genes of the obtained samples (5) were sequenced. As the origin of the patients is widely distributed over central, western and southern Sudan, the disease seems to occur sporadically in a large part of the country. All of five cysts samples were E. canadensis G6. It was suggested that this strain may have a lower pathogenicity to humans due to its sporadic occurrence. This is especially so, as all epidemiological conditions for autochthonous transmission of CE are given: In rural areas there are large numbers of dogs in and around villages, and infection can occur with offal from slaughterhouses or during unsupervised home slaughtering. Nevertheless, even if the parasite may have lower infectivity to humans, the infection can occasionally get established and progress to clinical CE and all samples from the patients in our study were viable and contained protoscolices.

E28

Ocular toxoplasmosis: an update

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Objective: Toxoplasmosis is a worldwide occurring parasitic zoonosis and an important cause of posterior uveitis. Here we describe the prevalence, demographics and clinical features in a large cohort of patients with ocular toxoplasmosis (OT).
Method: A retrospective review of the charts of 156 patients with OT examined between 1996 - 2010.
Results: Active OT was diagnosed in 156 patients (approx. 2% of all uveitis patients), 80 (51 %) females and 76 (49 %) males. The mean age at first ocular manifestation was 32 years (0-80) ±19. At presentation, active disease was unilateral in almost all cases (84%). Among the eyes with active disease, 58 (37%) presented with a peripheral focal retinochoroiditis, 70 (45%) demonstrated involvement of the posterior pole. Large lesions (>2 papilla diameters in extension) were seen in 16 (10%) of our patients. Congenital OT was diagnosed as most likely in 18 (12%), while the majority (72%) of OT infections were acquired. Younger age was the single significant predictor of macular involvement. Vitreous haze was detected in 98 (74%) cases during active OT. The macula was involved in 29 (23%) patients. The mean best corrected visual acuities at first and last visits were 0.6 (0.001-1.0) ± 0.4 and 0.7 (0.016-1.0) ± 0.4 respectively ($p<0.0001$). "Standard therapy" was based on oral clindamycin in 117 (87%) individuals and temporarily combined with systemic steroids in 77 (60%) patients. Ocular complications related to OT were observed including increased ocular pressure (>25mmHg) in six (4%) patients and three (2%) individuals suffered from retinal detachment (RD). Among these patients one RD (1%) was preceeded by necrotising retinitis.
Conclusions: OT is a common cause of posterior uveitis. The most common contributers to decreased vision in eyes with active lesions were inflammation and macular involvement.
Presenting author: M. Maenz

E29

Serosurvey and risk factors for *Toxoplasma gondii* in domestic ducks and geese in Lower Saxony, Germany

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Keywords: Toxoplasma gondii, risk factor, Anseriformes, serology

To estimate the prevalence of *Toxoplasma gondii* infection in ducks and geese, enzyme-linked immunosorbent assays (ELISA) were established based on affinity-purified *T. gondii* tachyzoite surface antigen 1 (TgSAG1) and used to examine duck and goose sera for *T. gondii* specific antibodies. Field sera from ducks and geese sampled in the course of a monitoring program for avian influenza were examined by this ELISA for antibodies to *T. gondii*. In 145/2534 (5.7%) of the ducks and 94/373 (25.2%) of the geese antibodies against TgSAG1 were detected. Seropositive animals were found in 20 of 73 submissions from the 61 duck farms and in 11 of 13 submissions from 13 goose farms. The seroprevalence within positive submissions from individual farms ranged between 2.2% and 78.6%. Farms keeping ducks or geese exclusively indoors were significantly less likely (odds ratio 0.05, 95% confidence interval 0.01-0.3) of harboring serologically positive animals as compared to farms where the animals had access to an enclosure outside the barn.

This project was funded by the German Bundesministerium für Bildung und Forschung (01 KI 0765) as part of the Toxonet01Network (National Research Platform for Zoonoses).

Poster Session – Wildlife and new or re-emerging zoonoses

W01

Coxiella burnetii and *Borrelia burgdorferi* sensu lato in ticks from Moldova and Germany

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Keywords: ticks, Q fever, Borrelia, Moldova, Germany

In Europe, the most important tick-borne bacterial diseases are Lyme disease and Q-fever. Lyme disease is caused by spirochetes of the *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s. l.) complex, while Q-fever is caused by the rickettsia *Coxiella burnetii* (*C. burnetii*). The main arthropod vectors of these pathogens are ticks of the genera *Ixodes (I.)* and *Dermacentor (D.)*.
Investigations of the last years showed that many of the tick-borne diseases are able to be transmitted as mixed infections. The ecology and distribution of Q-fever and Lyme disease are not explored enough on the territory of Germany and Moldova, while this region represents a good research model for examination of ecology of ixodid tick-transmitted disease foci in respect to anthropogenic stress conditions.
Therefore 282 ticks of different species (*D. marginatus* [N= 48], *D. reticulatus* [N= 37]*, I. ricinus* [N= 197]) were sampled at different places of Moldova in autumn 2010. In spring 2011 further 583 ticks (*D. marginatus* [N= 81], *D. reticulatus* [N= 78], *I. ricinus* [N= 355] and *Haemaphysalis inermis* [N= 24]) were sampled. Pools of these ticks (5–10 ticks per pool) were investigated by PCR for both agents. DNA of *B. burgdorferi* s. l. was detected in 31 pools of *I. ricinus* and 1 pool of *D. marginatus*. All samples were negative for *C. burnetii*-specific DNA. The tick samples from Germany (Lower Saxony) are still under investigation.

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W02

Tick-borne encephalitis and Borrelia in wildlife habitats and domestic animals

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Keywords: ticks, TBE, Borrelia, antibody, ELISA.

Tick-borne Encephalitis Virus (TBEV) is an arbovirus belonging to the Flavivirus family. *Borrelia* is a bacterium that belongs to the Spirochetes family and is also transmitted by tick bites.
Cases of infection by TBE in a horse and a dog with clinical symptoms have already been reported in literature.
In order to estimate the risk of infection by tick borne pathogens for humans living in these certain areas of the federal states of Germany and Belgium we analysed different domestic and wild animals for antibodies against TBE and Borrelia.
To generate positive control sera to be used for the evaluation of the commercial ELISA (Euroimmun®) for human sera, we have vaccinated horse, cattle and sheep with the TBE vaccine. The analysis of these sera shows a clear sero-conversion four weeks post vaccination.
In the study, the prevalence of TBE infection varied in relation to the animals tested. The sera were examined using the TBE IgG ELISA:
617 horse sera (28% positive of which 31% were from endemic areas); 154 dog sera (36% positive of which 33% were from endemic areas); 315 cattle sera (3% positive); 297 sheep sera (28% positive); 244 goat sera (no positives); 239 wild boar sera (19% positive); and 499 deer sera from Belgium (12% positive).
Borrelia burgdorferi and *afzelii* shown by ELISA and Immunfluorescence assay IgG from Euroimmun®: 90 horse sera (72% positive) and 186 deer sera from Belgium (43% positive).
Samples of wildlife brain tissue will be examined by PCR for TBE virus genome.

W03

Toxoplasma gondii prevalence and genotypes in red foxes (*Vulpes vulpes*) originating from different regions of Germany.

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Keywords: *Toxoplasma gondii*, PCR-RFLP, genotype, isolation, virulence

Toxoplasma gondii is a protozoan parasite that infects a number of warm-blooded vertebrates, including humans. Wild and domestic felids act as definite hosts that shed the oocyst stage which becomes infectious after sporulation. Intermediate hosts such as rodents, foxes, livestock animals, birds and humans become infected by either taking up oocysts or by feeding on infected intermediate hosts. *T. gondii* has a clonal population structure. In Europe, three clonal lineages of *T. gondii*, referred to as clonal types I, II and III, are observed. Previous studies predominately found type II oocysts in feline faecal samples from Germany. In this study we analysed 205 foxes from Brandenburg and 19 foxes from North Rhine-Westphalia for *T. gondii* antibodies and the presence of *T. gondii*-DNA in heart and lung tissue. The seroprevalence in foxes ranged between 78% and 88%. Only 17% of seropositive foxes were shown to be PCR-positive for *T. gondii*. The majority of foxes contained *T. gondii* DNA displaying type II alleles. However, *T. gondii* Type III- and recombinant allele patterns could also be observed. This is the first study on *T. gondii* genotypes in foxes from Germany. It confirms that foxes act as a reservoir for *T. gondii* and that clonal type II is the major, but not exclusive genotype found in German red foxes.

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W04

Red fox – the source of emerging zoonoses, alveolar echinococcosis and trichinellosis, in Slovakia.

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Keywords: zoonoses, *Echinococcus multilocularis*, *Trichinella* spp., red fox, Slovakia

Due to specific geographical localization and climatic conditions, several serious zoonotic diseases circulate in the territory of Slovakia. Red fox (*Vulpes vulpes*) is the most abundant wild carnivore living in Slovakia and suitable indicator species of two important parasitozoonoses – alveolar echinococcosis, caused by fox tapeworm *Echinococcus multilocularis* and trichinellosis.
The first systematic investigation of red foxes for parasites present initiated in 2000. Between 2000 and 2010 a total of 4761 red foxes were investigated for *E. multilocularis* and 5270 individuals for trichinellosis. *E. multilocularis* was found in small intestines of 1441 (30.3 %) foxes with mean worm burden of 1777 tapeworms. During the whole study period the highest prevalence and the highest infection intensity were recognized in northern regions of Slovakia; mean prevalence in several districts of these regions fluctuated between 40.0 % and more than 60.0 %. Larvae of *Trichinella* spp. were recovered from the muscles of 608 foxes (11.5 %). Significant increase of prevalence was recognized during the studied period. The highest number of infected foxes was recorded in Eastern and Central Slovakia. The larval burden varied from 0.06 to 283 lpg. *Trichinella britovi* was the most frequent etiological agent (98.8 %) of sylvatic trichinellosis in Slovakia. Two animals harboured *T. spiralis*, 2 mixed infection with *T. britovi* and *T. spiralis* and in 1 specimen *T. britovi* and *T. pseudospiralis* co-infection was confirmed.
The work was realised within frame of the project "Aplikačné centrum pre ochranu l'udí, zvierat a rastlín pred parazitmi" (Code ITMS: 26220220018) based on the support of the Operational Programme "Research and Development" funded from the European Regional Development Fund (0.8) and by the Science Grant Agency VEGA 2/0145/09.

W05

Canine dirofilariosis, new-found parasitozoonosis in the territory of Slovakia.

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Keywords: dirofilariosis, *D. repens*, *D. immitis*, dogs, Slovakia

Various global changes, particular global warming, vast floods and increase of "pets´ travelling activities" are considered to be main risk factors of vector-borne diseases spreading. In Central Europe, canine subcutaneous dirofilariosis caused by *Dirofilaria repens* is regarded as arthropod-born infection with the fasted spread.
In Slovakia, *D. repens* in dog was for the first time recorded in 2005; in 2007 the first epizootological survey of canine dirofilariosis was initiated. During three years nearly 1000 dogs from all regions of Slovakia were examined.
Great regional differences in prevalence were recorded: the majority of infected dogs came from southern regions of the country (Trnava 45.2 %, Nitra 31.4 %), the lowest prevalence of canine dirofilariosis was detected in northern part of Slovakia (Žilina region 2.2 %, Prešov region 7.0 %). The highest prevalence of dirofilariosis was recorded in shepherd and watch dogs (45.7 %) and hunting dogs (40.5 %). In the group of police dogs 20.5 % individuals were infected. The lowest prevalence was found among companion dogs (7.4 %).
Using PCR *D. repens* was detected in all infected animals; in seven animals co-infection with *D. immitis* (heartworm) was present. Autochthonous origin of infection has been confirmed in all infected dogs.

The work was realised within frame of the project „Ochrana životného prostredia pred parazitozoonózami pod vplyvom globálnych klimatických a spoločenských zmien" (Code ITMS: 26220220116) based on the support of the Operational Programme "Research and Development" funded from the European Regional Development Fund (0.8) and by the Science Grant Agency VEGA 2/0145/09.

W06

Susceptibility of bat cell lines to viral infections. Ready to enter?!

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Keywords: bats, species barrier, entry, receptor, zoonosis

Bats (order: *Chiroptera*) have been proposed or at least are in the focus as natural reservoirs for a variety of zoonotic viruses. Spillovers to humans and their associated animals (cattle, pigs, …) can occur if they get in close contact to infected bats or their excretions (droppings, saliva) and if the virus has the potential to cross the species barrier. A major barrier is encountered at the site of virus entry which depends on the presence of proper receptor molecules.
To investigate the species barrier and in particular the entry process we assess the potential of a broad spectrum of RNA viruses - most of which are able to infect different mammalian species/cell cultures - to enter bat cells. For this purpose, we analyze the susceptibility of different bat cell lines to infection by corona- (SARS and SARS-like CoV), rhabdo- (VSV), paramyxo- (SeV, BRSV), filo- (ZEBOV, MARV), and orthomyxoviruses (influenza A viruses) using either replication-competent virus or, in the case of the highly pathogenic viruses, a pseudotype system based on a single-cycle vesicular stomatitis virus that expresses soluble eGFP and fLUC from two independent ORFs. We determined the infectibility by fluorescence microscopy and in the case of the VSVpp system also by a luciferase assay.

SARS CoV= severe acute respiratory syndrome associated coronavirus; VSV = vesicular stomatitis virus; SeV = sendai virus; BRSV = bovine respiratory syncytial virus; ZEBOV = zaire ebolavirus; MARV = marburgvirus; eGFP = enhanced green fluorescent protein; fLUC = firefly luciferase; ORF = open reading frame

W07

Detection of Paramyxovirus RNA in European Bats

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Keywords*:* bats, paramyxoviruses, population survey

Flying foxes (fruit bats, *Macrochiroptera*) have been identified as subclinical carriers of Nipah and Hendra viruses belonging to the family of *Paramyxoviridae* in South-East Asia and Africa. Serological evidence for henipavirus infection in insectivore bats (*Microchiroptera*) exists for one Asiatic species, the lesser asiatic yellow house bat (*Scotophilus kuhlii).* Since these agents may cause fatal diseases in humans and animals, we are interested to assess whether members of the paramyxovirus family are also present in European insectivore bats. For this purpose oral swab and urine samples were collected in the course of population surveys of bat colonies across Germany. Using a published pan-paramyxovirus PCR protocol, we were able to amplify nucleotide sequences from the L-gene suggesting that a novel paramyxovirus circulates in the soprano pipistrelle (*Pipistrellus pygmaeus)* and the greater mouse eared bat (*Myotis myotis*). These sequences revealed a phylogenetic relation to rodent associated paramyxoviruses like J-virus, Mossman virus and Nariva virus from Trinidad and Tobago but also to Beilong virus, which was first isolated from a human kidney mesangial cell culture in China. No obvious clinical signs were observed in bats tested positive and no associated viral infections were reported in activists involved in bat colony surveys to date. Future studies at FLI will address the questions regarding the epidemiological and zoonotic relevance of this newly discovered bat virus.

W08

An insect nidovirus emerging from a primary tropical rainforest

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Keywords: nidovirus, emerging virus, mosquito, rainforest

Tropical rainforests show the highest level of terrestrial biodiversity and may be an important contributor to microbial diversity. Exploitation of these ecosystems may foster the emergence of novel pathogens.
We report the discovery of the first insect-associated nidovirus, tentatively named Cavally virus (CAVV). CAVV was isolated with a prevalence of 9.3% during a survey of mosquito-associated viruses along an anthropogenic disturbance gradient in Côte d´Ivoire. Analysis of habitat-specific virus diversity and ancestral state reconstruction demonstrated an origin of CAVV in pristine rainforest with subsequent spread into agriculture and human settlements. Virus extension from the forest was associated with a decrease in virus diversity ($p<0.01$) and an increase in virus prevalence ($p<0.00001$).
CAVV are enveloped viruses with large surface projections. The RNA genome comprises 20.108 nt with seven major ORFs. ORFs 1a and 1b encode two large proteins that share essential features with phylogenetically higher representatives of the order *Nidovirales*, but also with families in basal phylogenetic relationship. Genetic markers uniquely conserved in nidoviruses, such as endoribonuclease and helicase-associated zinc-binding domain, are conserved in CAVV. ORFs 2a and 2b are predicted to code for the structural proteins S and N. ORFs 3a and 3b encode proteins with membrane-spanning regions. CAVV produces three subgenomic (sg) mRNAs with 5'-leader sequences of different length. In addition we detected two further nidoviruses (A4/CI/2004 and F24/CI/2004) in the mosquitoes showing only 76-84% identity to CAVV. This novel cluster of mosquito-

associated nidoviruses is likely to represent a novel family within the order *Nidovirales*.

W09

Hepatitis E virus infections in pigs and wild boars – an experimental study

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Keywords: Hepatitis E virus, HEV, wild boars, pigs

Hepatitis E virus (HEV) is a causative agent of acute clinical hepatitis in humans, primarily in developing countries. However, an increasing number of autochthonous HEV infections have been found in industrialized countries recently. Human isolates belong to HEV genotypes 1, 2, 3 and 4. Genotype 1 and 2 are exclusively detected in humans, whereas genotypes 3 and 4 seem to represent also zoonotic viruses with domestic pig and wild boar being reservoir hosts. Cases of hepatitis E in humans have been linked to the consumption of undercooked meat products of the infected animals by HEV genotype 3.

The aim of the present study was to investigate the propagation of wild boar-derived HEV (genotype 3) in pigs and wild boars after intravenous inoculation. Additionally, sentinel animals were included in the approach to detect horizontal transmissions. Blood and faecal samples of infected animals and sentinels were collected periodically and analyzed to determine the onset of viremia, faecal HEV excretion and seroconversion. After necropsy (28/29 dpi) tissue samples were taken for the detection of HEV RNA using an in-house qRT-PCR and for histopathological examinations. Data were used to identify differences in the outcome and pathogenesis of HEV infection in wild boars and domestic pigs after intravenous or faecal-oral inoculation.

Results demonstrate for first time that wild boar-derived genotype 3 HEV can be transmitted easily to domestic pigs by intravenous inoculation. Moreover, we describe the horizontal transmission between wild boars and also from wild boar to domestic pigs as a possible origin for HEV infections.

Poster Session – Functional genomics

F01

Role of putative RNA-binding proteins in *Campylobacter jejuni* gene expression and virulence

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Keywords: *Campylobacter jejuni*, RNA-binding proteins, colonization, gnotobiotic mice, IL-10-deficient animals

RNA-binding proteins like the RNA chaperone Hfq exert global control of different forms of post-transcriptional regulation of gene expression in many bacteria, by facilitating the interaction between small RNAs and mRNAs. While the epsilon-Proteobacteria *Campylobacter* and *Helicobacter* do transcribe small RNAs, they do not have a recognisable Hfq ortholog. Using bioinformatic approaches, we have identified 3 genes (Cj0138, Cj0667 and Cj1103) in the *C. jejuni* genome which encode proteins with a predicted RNA-binding motif, and have investigated their role in gene expression and colonization/virulence using gnotobiotic IL-10 deficient mice.
Mutant strains were generated with an inactivated gene, with two copies of the gene, and one where the inactivated gene was complemented *in trans*. Growth of the mutants was similar to that of the wild-type strain, but while inactivation of Cj1103 did not affect motility or growth phenotypes, overexpression of Cj1103 resulted in a significant decrease in autoagglutination (AAG). Transcriptomic and proteomic characterisation of these strains suggested changes in expression of flagellar genes, which may explain the AAG phenotype.
Infection of gnotobiotic IL-10 deficient mice with the *C. jejuni* wildtype strain caused acute bloody diarrhea, histopathology and inflammatory responses in the colon. All mutants colonized the mouse intestine to similar levels as the wildtype strain, but differed in their potential to induce diarrhea and inflammatory responses in the colon. Clinical signs of disease and levels of pro-inflammatory cytokines in the colon were significantly reduced in the Cj0138 mutant when compared to the wildtype strain.

Although there are subtle phenotypes associated with these putative RNA-binding proteins, there is currently no proof that these proteins function as general RNA-chaperones, as the predicted pleiotropic phenotypes were not observed. The results obtained in the infected gnotobiotic IL-10-deficient mice indicate a role of the genes Cj0138, Cj0667 and Cj1103 in *C.jejuni* virulence.

F02

Comprehensive genome and phenotypic characterization of closely related *Campylobacter jejuni* strains from different sources

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Campylobacter jejuni and *Campylobacter coli* are important intestinal pathogens of humans worldwide. Anthropozoonotic transmission from life stock animals or animal-derived food is the likely cause for most of these infections. In certain animal hosts, *Campylobacter* species colonize persistently and do not cause disease, while they frequently cause acute intestinal disease in humans. However, little is known about host-specific colonization factors.

We have addressed the question of possible host-specific properties using phenotypic characterization and genome-wide analysis of genetically closely related *C. jejuni* strains from different sources.

From our recent collection of over 500 *Campylobacter* isolates (FBI-Zoo *Campylobacter* collection), which was characterized via MLST, we have chosen a subset of closely related *C. jejuni* strains of sequence type (ST) 21 from different hosts and isolation sources. PCR typing of strain-variable genes provided first evidence that some genes differed between these strains. Furthermore, the phenotypic variation of these strains was tested using the following criteria: metabolic variation, protein expression patterns and eukaryotic cell interaction and activation. The results provided clear evidence for phenotypic diversity within the ST21 group. Whole genome sequencing and comparison were performed for five strains from the ST21 group from bovine, chicken, human and food sources. This approach was also aimed at answering the question, whether the observed strain diversity

correlated to a specific host and connected to a host-specific genetic set-up.
Taken together, the data obtained in vitro and in a chicken infection model provided little evidence of fixed adaptation to a specific host. Rather, *C. jejuni* appears to be characterized by phenotypic flexibility and high genetic microdiversity, revealing properties of a generalist. These are supposed to enable the bacteria to rapidly and reversibly change their properties, allowing *C. jejuni* to flexibly express diverse fitness factors in changing environments.

F03

Regulative networks in *Salmonella* infections of piglets

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Keywords: microRNA, pig, salmonella infection

Infections of piglets during weaning can be fatal and thus lead to increased mortality. In this period, the protective effect of colostrum is missing and piglets are exposed to new antigens. Furthermore, this period is important for intestinal development. Recent studies identify microRNAs as key regulators of intestinal development and immunological processes showing that their dysregulation leads to intestinal malignancy.
The aim of this study was the integrated analysis of the porcine intestinal mRNA as well as microRNA expression during the postnatal development with regards to *Salmonell*a Typhimurium infection as well as *Salmonella* Typhimurium / *Enterococcus feacium* co-treatment.
Gene expression analyses were conducted using the microarray-platform Pigoligoarray [1] while microRNA expression was studied using customized Geniom biochips (*febit*). Pathway analyses were carried out using the web based tool *DAVID* [2]. Integrated analysis of both datasets was performed using the web based tool *MAGIA* [3], microRNA-target interaction were visualized as networks using *Cytoscape* [4]
Our results showed mRNAs as well as microRNAs, which were significantly dysregulated upon *Salmonella* infection. Affected pathways were involved in signaling in immune system, pathogenic *E. coli* infection, apoptosis, metabolism of proteins and integration of energy metabolism, respectively. The integrative analysis of mRNA and microRNA expression allowed us to provide for the first time a theoretical concept of regulative networks in *Salmonella* infections of piglets.
[1]www.pigoligoarray.org [2]http://david.abcc.ncifcrf.gov/
[3]http://gencomp.bio.unipd.it/magia [4]www.cytoscape.org
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F04

Gene expression analysis of the innate mucosal immune response to *Salmonella enteritidis* in the chicken

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Keywords: chicken, Salmonella, innate immune response

Poultry and poultry products are major sources of *Salmonella* infections in humans. However, *Salmonella* species with zoonotic potential do not cause diseases in chickens despite their ability to infect the gut mucosa and to spread to liver and spleen in small numbers. To get a better understanding of the immune response to *Salmonella enteritidis* infection and potential control mechanisms, we investigated gene expression patterns in ceacum and ceacal tonsils of 8 weeks old white leghorn chickens up to 48 hours after oral infection. Tissue samples were subjected to gene expression analysis using a 4x44K chicken microarray (Agilent).
Numbers of differentially expressed genes peaked in ceacal tissue and ceacal tonsils at 12 hours post infection. However, the response observed in caecal tonsils was unexpectedly weak whereas more than 6500 genes were differentially regulated in the caecal tissue. A more detailed analysis of the latter samples revealed that 41 signalling pathways were significantly regulated at 12 hours post infection. As expected, Th1 cytokines (e.g. IL-12p40, IL-18, IFN-γ), inflammatory cytokines (e.g. IL-1, IL-6, TLA1) and several chemokines (e.g. CCL4, CCL20, CXCL8, K60) were strongly induced. Our results indicate that the early inflammatory response plays an important role in limiting bacterial invasion. Rapid shut down of this response characterised by reduced numbers of differentially expressed signalling pathways at later time points and the induction of IL-10 and IL-10R expression may explain absence of clinical symptoms in infected birds. Candidate genes identified in this study will be cloned and functionally analysed *in vivo*.

F05

De novo sequencing of the complete genome of *Chlamydophila psittaci* type strain 6BC

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Keywords: *Chlamydophila psittaci*, next-generation sequencing, intracellular bacteria

Chlamydophila (C.) psittaci is an obligate intracellular Gram-negative bacterium. It infects primarily birds, but has a wide range of secondary hosts, e.g. cattle, horse, and goat. A zoonotic potential is known.
C. psittaci causes psittacosis with diverse clinical symptoms, ranging from no or mild clinical signs, like headache and cough, up to sepsis with multi-organ failure, and death. [1]

We report the full length genome sequence of the *C. psittaci* strain 6BC, originally isolated from a parakeet.
Using a combination of 454 pyrosequencing, Illumina- and Sanger Sequencing the complete genomic sequence was identified.
The *C. psittaci* 6BC genome consists of a circular chromosome of 1.17 million bp and a plasmid of 7553 bp.
The genome was assembled using MIRA3 [2] and annotated using the ISGA prokaryotic annotation pipeline [3].
For the bacterial chromosome and the plasmid, 967 and eight protein-coding genes were predicted, respectively. The chromosome of *C. psittaci* 6BC has a GC content of 39.06%. It encodes for 38 tRNA genes and one ribosomal RNA operon.

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F06

T. gondii and *N. caninum*: Identification and characterisation of the homologue protein GRA9

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Keywords: *Neospora caninum,* dense granule proteins

Neospora caninum is an obligate intracellular apicomplexan parasite which is very close related to *Toxoplasma gondii*. The definitive hosts of *N. caninum* are dogs and cattle display the main intermediate hosts. This work deals with dense granule proteins which are known to have important roles in host-parasite-relationship. Previously, we described the dense granule protein GRA9 in *T. gondii.* In this study we identified the putative homologue in *N. caninum*.
The genomic sequence of *Nc*GRA9 is located on chromosome XII and consists of two exons. By Southern Blot it was proven that it is present as a single copy gene. After production of a *N. caninum* specific anti-GRA9 antiserum against the recombinant protein it could be shown by Western Blot that the *Nc*GRA9 gene encodes a 40,2 kDa protein. Furthermore, we defined *Nc*GRA9 as a member of the excreted-secreted antigens (ESA) of *N. caninum*. By confocal microscopy it was observed that in extracellular parasites the protein is accumulated in a dotted pattern which points out that *Nc*GRA9 is located in the dense granule organelles. Furthermore, we could show that after invasion of the parasites into the host cell *Nc*GRA9 is secreted into the parasitophorous vacuole (PV). Thus *Nc*GRA9 fulfils all main characteristics of GRA proteins.
In sum we could verify in this study that *Nc*GRA9 and *Tg*GRA9 are structurally similar, show immunological cross reactivity and are similar distributed within the respective parasites which indicate a close relationship between both proteins.

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F07

Characterization of yellow fever virus NS4B charged-to-alanine scanning mutants

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Keywords: yellow fever virus, reverse genetic, charged-to-alanine scanning, NS4B

The positive stranded RNA genome of yellow fever virus (YFV) encodes one large polyprotein, which is cleaved co- and posttranslationally by cellular and viral proteases to yield three N-terminally located structural proteins followed by seven nonstructural proteins (NS).
For the YFV NS4B protein, charged-to-alanine scanning was performed. The resulting mutants were analyzed using both a reporter gene expressing YFV replicon and a YFV full-length cDNA clone. The majority of mutations resulted in impaired replication efficiencies. Cells electroporated with mutants initially exhibiting a more or less lethal phenotype were passaged in order to obtain compensating second site mutations. For two charged-to-alanine scanning variants, second site mutations could be obtained in NS4B and NS2B, respectively. Reconstruction of the recovered amino acid exchanges in the context of the original mutants using the available reverse genetic systems confirmed the compensating nature of the second site mutants. The fact that a mutation in NS2B could compensate for a defect in NS4B suggested that the two proteins interact, which was supported by coimmunoprecipitation analyses. However, coimmunoprecipitation analyses of wild type and mutant NS4B with wild type and mutant NS2B could not demonstrate a direct correlation between protein-protein interaction and the compensatory effect.
In addition, since NS4B has been described to function as interferon antagonist, analyses of viable mutants in interferon deficient cells (BHK) versus interferon competent cells (A549) were performed. One mutant could be identified, which replicated about 100 fold less efficient in A549 cells supporting a role of NS4B as interferon antagonist.

F08

Pandemic H1N1 influenza virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness *in vivo*

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Keywords: neuraminidase inhibitor, resistance, zanamivir, viral fitness

Neuraminidase inhibitors are routinely used for the treatment of severe influenza A virus infections. Oseltamivir resistance became widespread in seasonal H1N1 influenza viruses in 2007/2008, and oseltamivir-resistant pandemic H1N1 viruses (pH1N1) also seem to retain a high degree of fitness. Zanamivir resistance is observed only infrequently in influenza viruses and little is known about the ability of pH1N1 to tolerate resistance mutations.
In this study we identified a glutamine-to-lysine mutation at position 136 (Q136K) of the neuraminidase which confers zanamivir resistance. The mutation was acquired during passage in MDCK cells in the presence of increasing concentrations of zanamivir. Introduction of this mutation into the wild-type virus by reverse genetics resulted in enhanced resistance against zanamivir in a neuraminidase activity assay. The oseltamivir resistance of this mutant was not increased. Growth kinetics on MDCK cells revealed no significant differences between the mutant (rHH-Q136K) and the wild-type virus (rHH-wt). However, when growth kinetics experiments were performed on MDCK cells over-expressing α-2,6-sialic acids (MDCK-SIAT), replication of rHH-Q136K was markedly delayed as compared to rHH-wt. Guinea pigs experimentally infected with rHH-Q136K had only moderate viral titres in nasal washings and did not transmit the virus to contact-exposed animals.
Our data shows that pandemic H1N1 viruses may gain zanamivir resistance under vigorous selective pressure. However, such neuraminidase mutations seem to result in dramatic losses in viral fitness. Therefore, it is unlikely that zanamivir-resistant mutants of pH1N1 become widespread in the human population.

F09

To master one's fortune – the generation of a complete library of Cowpox virus knock-out mutants.

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Keywords: Cowpox virus, BAC, transposon mutagenesis, host range

Because of the termination of comprehensive vaccination against Variola using Vaccinia viruses some 30 years ago, the number of seronegative individuals has increased, as has the risk of acquisition of zoonotic orthopoxvirus (OPV) infections. Depending on the virulence potential of the infecting OPV, such infections can have high clinical impact. Cowpox virus (CXPV) harbors the most complete genome among members of the genus. The virus has a known zoonotic potential, shows a wide host range, and yet virus isolates exhibit strikingly different virulence patterns.
Bacterial artificial chromosomes are versatile tools to modify large virus genomes in Escherichia coli. We generated a full length clone of the CPXV strain Brighton Red (BR) termed pBRF. From BAC DNA we could reconstitute virus (vBRF) in chicken embryo cells using Rabbit fibroma virus as helper virus. Thus we have a tool at hand which allows all kinds of markerless, targeted, or random sequence modification without any selection for viral function.
This BAC clone shall be used to determine viral factors which are needed for the wide in vitro host range of CPXV. For this purpose we started to generate a complete library of defined knock-out mutants for every unique gene in pBRF. A specific real time PCR based assay shall be used to select dedicated clones from a random set of transposon mutants. The knock-out library will be used in our high throughput assay to characterize the in vitro host range of the derived virus mutants.

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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.

All researchers working on zoonoses in Germany are welcome to join the National Research Platform for Zoonoses.

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 which support interdisciplinary exchange and interaction.
- Encouragement of communication as well as national, European and international collaboration.
- Registration, harmonization and standardization of existing resources, including the setting up of both real and virtual specimen databases.
- Providing information about zoonotic infectious diseases for the general public.
- Initiation and realization of innovative and interdisciplinary pilot projects with cross-sectional character.

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 it is necessary to collaborate interdisciplinaryly. Networking within research consortia gets more and more important. To encounter the challenge of zoonotic infectious diseases, the National 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 are organised, where specific topics are presented and discussed. During 2011, four successful workshops were organized by the National Research Platform for Zoonoses.

For further information please visit our website www.zoonosen.net.

National Symposium on Zoonoses Research 2011

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