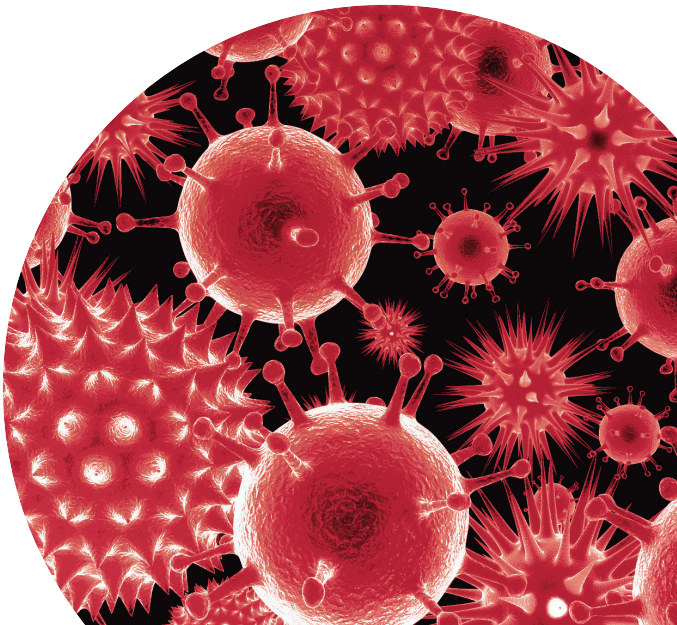
Nationale
Forschungsplattform
für Zoonosen

# National Symposium on Zoonoses Research

7 - 8 October 2010 | Berlin

2010

## Programme and Abstracts



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Nationale
Forschungsplattform
für Zoonosen

Editor
National Research Platform for Zoonoses

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# Welcome Address of the National Research Platform for Zoonoses

Dear colleagues,

Welcome to Berlin and to the National Symposium on Zoonoses Research 2010.
The symposium is now held for the fourth time and has without doubt developed into an important forum for zoonoses researchers in Germany.
From new and re-emerging infectious diseases up to risk assessment, diagnostics and many other topics – this symposium reflects the broad field of zoonoses research in Germany. Six internationally renowned speakers will give plenary talks on current issues such as the West Nile epidemic in South Eastern Europe or parasitic zoonoses in Central Europe – just to mention two examples. Many thanks to all plenary speakers for their contribution to the National Symposium on Zoonoses Research 2010!
We also would like to thank all of the authors of submitted abstracts for their enthusiastic participation. Your contributions, over 150 abstracts in total, will ensure that this meeting will be again a most productive one. Given the large numbers of abstracts, it is inevitable that all excellent submissions could not be accommodated as oral presentations, and abundant exceptional research will be found within the poster session. We would therefore encourage participants to pay close attention to the poster sessions as well.
One of the special aims of this years' symposium is to give support to young researchers: They are particularly invited to present their work and to meet colleagues from all over Europe, for example in session 8 "Junior scientists meet senior scientists". This year, the selection of abstracts especially focused on contributions of junior scientists, and we decided to award a poster prize for the first time.

We hope that you will enjoy the programme and that you will have stimulating discussions with colleagues and friends eventually resulting in new co-operations and networks.

| S. Ludwig | M. H. Groschup | S. C. Semler |
|---|---|---|
| (Münster) | (Greifswald – Insel Riems) | (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 2010

Bei fast allen neuen Erregern der vergangenen Jahre, so bei SARS, der „Vogelgrippe" und zuletzt bei der „Schweinegrippe", handelt es sich um Zoonosen – also um Erreger, die vom Tier auf den Menschen übertragen werden. Diese Infektionskrankheiten haben in den zurückliegenden Jahren stark zugenommen.

Um diese Krankheiten besser vermeiden und bekämpfen zu können, müssen Forscher und Mediziner die Krankheitsentstehung und den Verlauf besser verstehen. Eine der wichtigsten Grundvoraussetzungen dafür ist die enge Kooperation von Vertretern aus der Human- und Veterinärmedizin. Diese interdisziplinäre Herangehensweise ist im Bereich zoonotischer Infektionskrankheiten ganz besonders effizient.

Das Bundesministerium für Bildung und Forschung hat diese Herausforderung bereits 2006 erkannt und stellte für die Zoonosen-Forschung seither rund 30 Millionen Euro zur Verfügung. Im Zentrum der in interdisziplinären Forschungsverbünden geförderten Projekte steht die wissenschaftliche Zusammenarbeit aller Partner. Denn nur so wird jene wissenschaftliche Basis geschaffen, die die Basis zur Verbesserung der Prävention, Diagnostik und Therapie der zoonotischen Infektionskrankheiten bildet.

Mit Beginn der zweiten Förderphase werden vier zusätzliche Verbünde ihre Arbeit aufnehmen: zwei Verbünde zu vernachlässigten zoonotischen Infektionen und zwei Verbünde zu Bakterien mit Antibiotika-Resistenzen, die vom Tier auf den Menschen übertragen werden. Damit weitet das BMBF sein Engagement erheblich aus.

Um einen regelmäßigen wissenschaftlichen Austausch der Zoonosenforscher in Deutschland zu ermöglichen, hat das BMBF federführend ein jährliches Treffen der Wissenschaftler in Berlin initiiert. Im September 2007 trafen sich die Zoonosenforscher erstmals. Seit 2009 erfolgt die Organisation des Symposiums durch die Geschäftsstelle der Nationalen Forschungsplattform für Zoonosen, die ebenfalls vom BMBF gefördert wird.

Ich freue mich, dass das jährliche Treffen der Zoonosenforscher inzwischen zu einer festen Institution geworden ist und dieses Jahr – im größeren Rahmen als Symposium - wieder in Berlin stattfindet.

Ich wünsche allen Teilnehmerinnen und Teilnehmern eine erfolgreiche Veranstaltung mit fruchtbaren Diskussionen, bereichernden Begegnungen und tragenden Ergebnissen.

Joachim Krebser
Bundesministerium für Bildung und Forschung

## Grußwort des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz

Im Jahr 2006 haben sich die drei Bundesministerien BMBF, BMG und BMELV zum Ziel gesetzt, der Forschung über die vom Tier auf den Menschen bzw. umgekehrt übertragbaren Krankheiten eine noch größere Aufmerksamkeit zu schenken. Waren die fachlichen Beweggründe seinerzeit möglicherweise unterschiedlich, so bestand schon früh Konsens über die Notwendigkeit eines gemeinsamen Vorgehens: Um politisch notwendige Maßnahmen gegen Zoonosenerreger und zum Schutz von Tier und Mensch rasch und sachgerecht treffen zu können, bedarf es mehr, als die spezifischen Kenntnissen eines Fachbereichs, die den Zuständigkeitsbereich eines einzelnen Ressorts betreffen. Die Disziplinen müssen sich ergänzen, so wie sich die Zuständigkeiten der Ressorts ergänzen. Interdisziplinäre Forschung in einem Verbund ist der Schlüssel zu wissenschaftlichen Synergien, der Dialog der Disziplinen ist Voraussetzung, Sachverhalte aus verschiedenen Perspektiven zu beleuchten und so neue Lösungswege zu entwickeln.

Die wissenschaftlichen Ergebnisse der Zoonosenplattform aus den ersten Jahren zeigen deutlich: Die Bundesressorts haben die richtige Entscheidung getroffen! Diese ersten Ergebnisse zeigen aber auch, dass weiterhin enormer Forschungsbedarf besteht.

Zoonosenerreger stellen eine ständige Bedrohung für Mensch und Tier dar und besitzen erhebliche wirtschaftliche Auswirkungen auf die Tier haltende Landwirtschaft. Dies umso mehr im Zeitalter der Globalisierung. Die Auswirkungen auf den innergemeinschaftlichen und internationalen Handel sind zudem im Einzelfall unabsehbar.

Die hohe Mobilität der Menschen, zusammenwachsende Märkte mit weltweiten Handelsbeziehungen verbunden mit raschem Warenaustausch, verschärfen diese Gefahr. Diese Faktoren stellen die Politik und die Wissenschaft vor neue Herausforderungen. Zusammenhänge müssen rasch erkannt, effektive und effiziente Lösungswege erarbeitet werden. Die Globalisierung macht vor Zoonosen nicht halt – sie begünstigt sie. Geflügelpest

(„Vogelgrippe") und Schweinegrippe sind hierfür bekannte Beispiele.

Der Schutz der Tierbestände, die berechtigte Forderung der Verbraucher nach sicheren Lebensmitteln verbunden mit der Wahrung der deutschen Exportinteressen, erfordern mehr denn je ein besseres Verständnis der epidemiologischen Zusammenhänge und machen es notwendig, neue Schutzstrategien zu entwickeln.

Dies war auch Anlass für das BMELV, selbst weitere Mittel von mehr als 10 Mio. Euro zur Erforschung von Tierseuchen mit zoonotischem Potential zur Verfügung zu stellen. Als wichtiges Beispiel sei hier das interdisziplinäre Forschungssofortprogramm Influenza angeführt. Hier steht naturgemäß die Gesundheit der Tierbestände, indirekt aber auch die des Menschen im Vordergrund. Diese interdisziplinären Projekte finden sich natürlich ebenfalls auf der Zoonosenplattform.

Auch die Europäische Gemeinschaft ist sich im Jahr 2007 dieser Bedeutung bewusst geworden und hat die „One Health Strategy" ins Leben gerufen. Hierunter verbirgt sich eine neue Tiergesundheitsstrategie für die Europäische Union (2007-2013) „Tier + Mensch = Eine Gesundheit". Auch sie legt einen Schwerpunkt auf die Zoonosenbekämpfung, wobei alle Ebenen, von der Primärproduktion über den Schlachthof bis zur Lebensmittelproduktion und den Verbraucher einbezogen werden.

Im Rahmen der Beratungen zur „One Health Strategy" werden in den nächsten Jahren sicherlich viele wissenschaftliche Fragen zu lösen sein – Grund genug für die deutsche Forschung und Wissenschaft sich noch stärker als bisher als interdisziplinärer Verbund international zu positionieren.

Ich wünsche allen Teilnehmern eine erfolgreiche Tagung – nutzen Sie die Zoonosenplattform als Kommunikationsplattform! Erfolgreiche Projekte und innovative Ideen sind beste Argumente für eine Fortführung der nationalen Zoonosenforschung.

Dr. Hans-Joachim Bätza
Leiter des Referates ‚Tiergesundheit' im BMELV

## Grußwort des Bundesministeriums für Gesundheit

Die voranschreitende Globalisierung und der Klimawandel sind wesentliche Gründe für die zunehmende Bedeutung von Zoonosen als Gesundheitsrisiko. Dabei kommt den in den letzten Jahren und Jahrzehnten neu aufgetretenen Krankheitserregern, die vom Tier auf den Menschen übertragen werden, eine besondere Rolle Zu. Beispiele hierfür sind das Auftreten des SARS Coronavirus, Ebola Virus und Marburg Virus. Dabei kommt Krankheitserregern, die von nicht-menschlichen Primaten stammen eine besondere Relevanz, wie die Erkrankungen durch HIV und Ebola Virus verdeutlichen, zu. Obwohl diese Erreger in der Regel in entlegenen Gebieten der Erde mit niedriger Infrastruktur den Sprung auf den Menschen gemacht haben, verdeutlicht auch das Auftreten SARS Coronavirus und H5N1, dass es sich bei Zoonosen nicht mehr nur um lokal begrenzte Krankheitsgeschehen handelt, sondern eine weltweite Bedrohung darstellen können.

Die Zusammenarbeit zwischen Human- und Veterinärmedizin im Bereich der von Tieren auf Menschen übertragbaren Krankheiten zu unterstützen, ist der richtige Weg, der zunehmenden Herausforderung durch Zoonosen zu begegnen. Die Bundesregierung hat mit der Nationalen Forschungsplattform für Zoonosen im Rahmen der Hightech-Strategie eine zukunftsorientierte Zusammenarbeit der Human- und Veterinärmedizin unter Einbeziehung der Rossortforschung geschaffen. Die Ministerien für Ernährung, Landwirtschaft und Verbraucherschutz, Bildung und Forschung und Gesundheit bündeln damit die nationalen Kompetenzen und Ressourcen und ermöglichen damit den Aufbau fachübergreifender Kooperationsstrukturen. Ziel ist es, die Forschung voranzutreiben und neue Vorsorgestrategien wie auch Maßnahmen zur Bekämpfung zu entwickeln.

Der wissenschaftliche Austausch ist ein wesentliches Merkmal von erfolgreichen Kooperationsstrukturen, wie die bisherige gelungene Arbeit der Nationalen Forschungsplattform für Zoonosen eindrucksvoll beweist. Die Plattform intensiviert die enge Kooperation zwischen biomedizinischer Grundlagenforschung,

Human- und Veterinärmedizin einerseits sowie universitärer und außeruniversitärer Forschung andererseits.

Das Nationale Symposium für Zoonosenforschung 2010 hat sich zum Schwerpunkt die Förderung und Beteiligung von Nachwuchsforschern gesetzt und leistet damit einen herausragenden Beitrag, jungen Akademikern die Möglichkeit zu geben, ihre Forschungsergebnisse vor internationalem Publikum präsentieren und diskutieren zu können. Zudem eröffnet sich mit dieser Fokussierung die Chance, das Forschungsfeld Zoonosen als einen zukunftsfähigen Bereich für den akademischen Nachwuchs darzustellen.

Deshalb freue ich mich darüber, dass Sie im Rahmen des Symposiums den erfolgreichen interdisziplinären Dialog fortsetzen und sich wichtigen Themen widmen und sie voranbringen. Ich wünsche Ihnen eine gelungene Veranstaltung und viel Erfolg bei der Umsetzung der gewonnenen Erkenntnisse.

Prof. Dr. Michael Kramer, MPH, MBA
Bundesministerium für Gesundheit

# Programme - Thursday 7 October 2010

09.00 - 16.30 **Registration**

**Plenary Session**

11.00 – 11.30 **Opening Ceremony**

11.30 – 12.10 **The future of tropical medicine research**
Rolf D. Horstmann, Hamburg, Germany

12.10 – 12.35 **Zoonoses – from research to risk assessment**
Andreas Hensel, Berlin, Germany

12.35 – 13.00 **The pandemic influenza A H1N1: expectations - surprises - communication**
Reinhard Burger, Berlin, Germany

13.00 – 14.00 **Lunch Break**

**14.00 – 15.30 Session 1-3**

**Session 1: New and re-emerging diseases**

**Network "Rodent-borne pathogens": identification of a novel hepatitis E-like virus in wild Norway rats (*Rattus norvegicus*) from Germany**
Paul Dremsek, Greifswald – Insel Riems

**The ups and downs of Hantaviruses: longitudinal studies in natural reservoirs in an altitude-climate-gradient in Bavaria**
Sandra Essbauer, Munich

**Distribution of mosquitoes and frequency of virus infection differ in disturbed and undisturbed rainforest areas**
Sandra Junglen, Bonn

**Sandfly fever virus activity in Central/Northern Anatolia, Turkey: first report of Toscana virus infections**
Manfred Weidmann, Göttingen

**Toscana virus-associated encephalitis is emerging north of the Alps**
Frank Hufert, Göttingen

**Session 2: Pharmacology, therapeutics and resistance**

**Selective attenuation of influenza A viruses by targeting the polymerase subunit assembly**
Martin Schwemmle, Freiburg

**Oseltamivir-resistant variants of the 2009 pandemic H1N1 influenza A virus are not attenuated in the guinea pig and ferret transmission models**
Peter Stäheli, Freiburg

**Killing activity and protective efficacy of recombinant γ-phage lysin and synthetic theta defensins against *Bacillus anthracis***
Wolfgang Beyer, Stuttgart

**Approaches to the antimicrobial treatment of persistent *Chlamydia psittaci* infections**
Katharina Wolf, Jena

**Extended-spectrum betalactamase producing Enterobacteriaceae in animals: what does the future hold for us?**
Christa Ewers, Berlin

**Session 3: Wildlife zoonoses**

**Wildlife from Germany as hosts of multiresistant E. coli**
Sebastian Günther, Berlin

**The influence of the environment on *Trypanosoma cruzi* and other trypanosomes in Panamanian fruit bats**
Veronika Cottontail, Ulm

**Strong and specific amplification of Coronavirus associated with parturition in a bat maternity roost**
Jan Drexler, Bonn

**Novel Adenoviruses in wild primates: high genetic diversity and evidence for zoonotic transmissions**
Bernhard Ehlers, Berlin

**Transmission of retroviruses in primate predator – prey systems**
Fabian Leendertz, Berlin

**15.30 - 16.30** **Poster Exhibition**

16.30 – 18.30 **General Assembly National Research Platform for Zoonoses**

- Annual report
- Election of the internal advisory board

**from 18.30** **Get-together**

# Programme - Friday 8 October 2010

**09.00 – 10.30 Session 4-6**

**Session 4: Epidemiology, modelling and risk assessment**

**The story with the stork or why are ecological relations regaining popularity in zoonoses research?**
Lothar Kreienbrock, Hanover

**Bayesian time-space analysis of *Echinococcus multilocularis*-infections in foxes**
Franz Conraths, Wusterhausen

**Phylogeny and migration of Tick-Borne Encephalitis viruses in Southern Germany**
Gerhard Dobler, Munich

**Detection of a new chlamydial agent in birds**
Konrad Sachse, Jena

***Chlamydiaceae* in sheep flocks in Central Germany**
Hannah Lenzko, Jena

**Session 5: Cellular pathogenesis**

**Characterization of evolved SARS-Coronavirus variants in a reservoir and dead-end host context**
Marcel Müller, Bonn

**Novel insights into the proteolytic activation of influenza virus and SARS-Coronavirus**
Stefan Pöhlmann, Hanover

**Crk adaptor protein expression is required for efficient replication of avian influenza A viruses and controls JNK mediated apoptotic responses**
Eike Roman Hrincius, Münster

**The different functions of PI3K during influenza virus replication**
Christina Erhardt, Münster

**NS reassortment of an H7-type HPAIV affects its propagation by altering the regulation of viral RNA production and anti-viral host response**
Stephan Pleschka, Giessen

**Getting it out: potential role of influenza A virus non-structual protein 1 (NS1) in viral mRNA export**
Melanie Franz, Berlin

**Session 6: Immunity and pathogen-host-interaction**

**Profiling human and animal antibody responses to zoonotic infections by C*hlamydia abortus* leads to detection of potential virulence factors**
Vera Forsbach-Birk, Ulm

**Identification and characterization of a novel regulator STM0029 which contributes to *salmonella* intracellular survival and resistance to antimicrobial peptides**
Heng-Chang Chen, Berlin

**Botulinum neurotoxin serotype D attacks neurons via two carbohydrate binding sites in a ganglioside dependent manner**
Andreas Rummel, Hanover

**Protective Toxoplasma gondii-specific T cell responses require the T cell-specific expression of protein kinase C-theta**
Gopala Nishanth, Magdeburg

**Inhibition of IFN-γ-mediated immune responses by *Toxoplasma gondii* in host macrophages can be reversed by histon deacetylase inhibitors**
Carsten Lüder, Göttingen

**10.30 – 11.30** **Poster Exhibition**

**11.30 – 13.00** **Session 5-8**

**Session 5 (continued): Cellular pathogenesis**

***Coxiella burnetii* type IV secretion substrate AnkG inhibits host cell apoptosis by modulating p32 activity**
Anja Lührmann, Erlangen

**Tracing host cell determinants in Chlamydia infection**
Marion Rother, Berlin

**Hantavirus-induced kidney dysfunction is caused by disruption of tight junctions in renal endothelial and epithelial cells**
Ellen Krautkrämer, Heidelberg

**Inhibition of IFN-beta induction by Tribec virus**
Martin Spiegel, Göttingen

**Dissection of molecular species barriers with inter-genotypic Lyssavirus chimeras**
Stefan Finke, Greifswald – Insel Riems

**Session 6 (continued): Immunity and pathogen-host-interaction**

**The cellular receptor for West Nile virus – interaction with integrins?!**
Katja Schmidt, Greifswald – Insel Riems

**Replication and cytokine induction of pandemic H1N1 and H5N1 influenza A viruses in a human lung explant model**
Viola Weinheimer, Berlin

**Highly pathogenic influenza virus infection of the thymus interferes with T lymphocyte development**
Oliver Planz, Tübingen

**Insufficient protection of vaccinated chickens against Egyptian escape mutants of highly pathogenic avian influenza H5N1**
Christian Grund, Greifswald – Insel Riems

**Inhibition of the RIG-I dependent signalling pathway by the influenza B virus NS1 protein**
Daniel Voss, Berlin

**A polymorphism in the hemagglutinin of a highly pathogenic H5N1 influenza virus determines organ tropism in mice**
Benjamin Mänz, Marburg

**Session 7: Infrastructure, methods and diagnostics**

**MLVAplus.net: online database and analysis tool for bacterial typing with MLVA, SNP and other categorical data**
Thomas Weniger, Münster

**A comprehensive next generation sequencing strategy for full-length genomes of influenza A**
Dirk Höper, Greifswald – Insel Riems

**Peptide microarray-based serologic strain-typing of *Toxoplasma gondii* in infected humans from Germany**
Pavlo Maksimov, Wusterhausen

**A single mixed *Toxoplasma gondii* oocyst sample from Germany consists of many *T. gondii* clones of different virulences, genetically distinct from the clonal types I, II and III**
Daland Herrmann, Wusterhausen

**Novel murine models to study *Campylobacter jejuni* infection**
Stefan Bereswill, Berlin

**Experimental poxvirus infection in common marmosets (*Callithrix jacchus*): a new primate model for orthopox virus infections**
Kerstin Mätz-Rensing, Göttingen

**Session 8: Junior scientists meet senior scientists**

**Interdisciplinarity is a main feature of zoonoses research. For young professionals in the area of zoonoses research there are many different possibilities to design their individual careers. This session offers the opportunity for young professionals to interview senior scientists about their individual professional careers.**
**Interview partners are:**
Andrea Ammon, ECDC, Stockholm
Matthias Kuhn, CONGEN Biotechnologie GmbH, Berlin
Lothar Wieler, FU Berlin

**This session will be moderated by:**
Marcel Tanner, Swiss Tropical and Public Health Institute, Basel

**13.00 – 14.00** **Lunch Break**

**Plenary Session**

14.00 – 14.45 **RIG-I like receptors: sensing and responding to RNA virus infection**
John Hiscott, Montreal, Canada

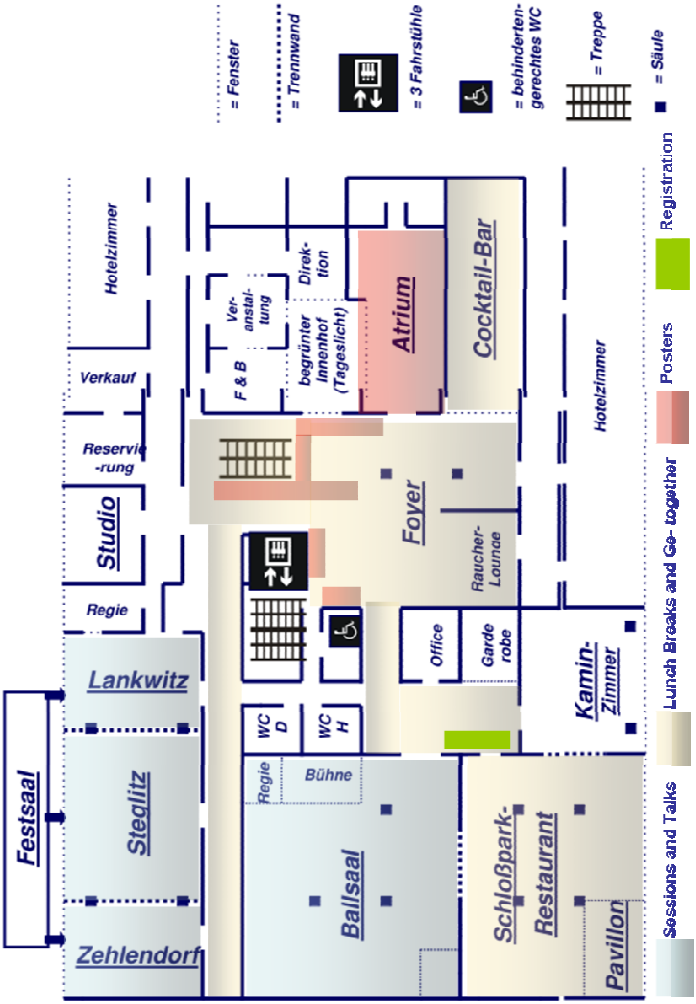
14.45 – 15.15 **Parasitic zoonoses in Central Europe – illusion or reality?**
Herbert Auer, Vienna, Austria

15.15 – 15.45 **Emerging viruses in Greece: Crimean-Congo hemorrhagic fever virus in 2008 – West Nile virus in 2010**
Anna Papa-Konidari, Thessaloniki, Greece

15.45 – 16.30 **Poster Awards**

16.30 **Closing Ceremony**

# Floor Plan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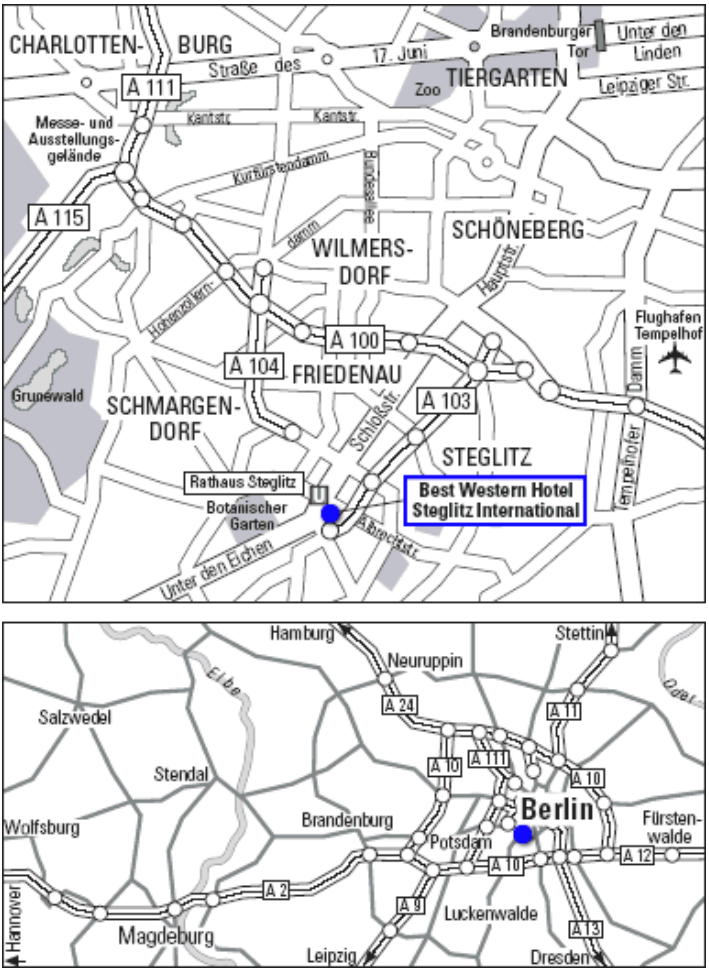
Atrium
Cocktail-Bar
Raucher-Lounge
Office
Garde robe
Kamin-Zimmer
Hotelzimmer
Schloßpark-Restaurant
Pavillon
Sessions and Talks
Lunch Breaks and Co- together
Posters
Registration

# Site Plan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Leipzig
Dresden

# 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 it´s work in spring 2009. Every researcher in Germany working in the field of zoonoses is invited to become a member within this network. Registration is possible via our website www.zoonosen.net.

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:

- Enhancing communication and collaboration on a national, European and worldwide level.
- Registration, harmonisation and standardisation of existing resources, including the set-up of databases.
- Providing information about zoonotic infectious diseases for the general public.
- Initiation and realisation of innovative and interdisciplinary pilot projects with cross-sectional character.
- Support and counselling for the design and implementation of zoonotic funding schemes.

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. Since the fight against zoonotic diseases needs interdisciplinary collaborations, 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 organises every year the National Symposium on

Zoonoses Research with more than 200 participants from all over Germany.

Furthermore, scientific workshops are organised, where specific topics are presented and discussed. During 2010, five successful workshops were held:

- Zoonotic and Vector-Borne CNS-Infections in Braunschweig,
- Neglected Diseases in Berlin.
- Elektronisches Meldewesen in Berlin,
- Ökologie und Speziesbarrieren bei neuartigen Viruserkrankungen in Bonn,
- Presenting Science (for PhD-students) in Berlin.

These workshops contribute to a better understanding of zoonoses, to the importance of infrastructure for zoonoses research and to the support and motivation of young researchers.

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

# Oral Presentations

# Session 1 – New and Re-Emerging Infectious Diseases

## Network "Rodent-borne pathogens": Identification of a novel hepatitis E-like virus in wild Norway rats (Rattus norvegicus) from Germany

P. Dremsek[1], A. Schielke[2], A. Plenge-Bönig[3], R. Petraityte[4], B. Köllner[5], E. Kindler[6], M.H. Groschup[1], S. Guenther[7], M. Heising[8], J. Reetz[2], G. Heckel[6], R. Johne[2], R.G. Ulrich[1]

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The network "Rodent-borne pathogens" facilitates the interdisciplinary collaboration between experts in rodent biology and pathogen research. Its major objectives are to explore the prevalence and evolution of rodent-borne pathogens as well as possible causes of outbreaks of rodent-transmitted diseases in the human host and to search for novel pathogens that may allow the development of disease or transmission models for human pathogens.
In this line, wild Norway rats from Hamburg were screened using a novel nested broad-spectrum RT-PCR protocol. The initial finding of hepatitis E virus (HEV)-like sequences in faeces was confirmed by the determination of the full-length genome from liver samples from rats of the same geographical origin. The genome organisation of this virus shows a typical genome organization of HEV with three major ORFs. The limited sequence similarities to human, mammalian and avian HEV and phylogenetic analysis of the nucleotide and deduced amino acid sequences suggest this virus to be a novel HEV genotype. For additional characterization of the virus the ORF-2-encoded capsid protein (CP) and derivatives thereof were expressed in *Escherichia coli* and yeast. A yeast-expressed truncated variant of the CP was found to form virus-like particles with morphology reminiscent of HEV. Future investigations have to examine the zoonotic potential of this virus and its possible application in an animal model for human hepatitis E.

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## The ups and downs of Hantaviruses: longitudinal studies in natural reservoirs in an altitude-climate-gradient in Bavaria

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In 2004 Puumalavirus (PUUV) carried by bank voles was found to cause a hantavirus outbreak in Lower Bavaria. So far there is a lack on longitudinal studies on hantaviruses in Germany. Hence in order to get an impression on the consistency, prevalence and occurrence of these agents we continued field studies in Bavaria. In 2008 we initiated a project in which rodents and associated pathogens are investigated along an altitude gradient ranging from 300m above sea level (asl) up to 1450 m asl in the Bohemian national park. Sampling sites were selected from the BIOKLIM-project in which many data on the biotopes are collected, e.g. several climatic data for comprehensive statistical analyses. In summary, so far approximately 500 rodents were collected since 2004, with 350 animals originating from the altitude-temperature gradient in the national park. Using hantavirus-specific assays we could show PUUV prevalences ranging from 2 to 30%. Rodent trapping indices and PUUV occurrence in bank voles seem to reflect the dynamics of human case reports. However, we also detected virus in regions where human infections have not been reported so far. Sampling and data collection will continue at least until 2011. In conclusion the results of this project will enable to get knowledge on the microevolution of PUUV, to find factors influencing the dynamics of focuses, and to perform a risk analysis for the region. This work is part of the VICCI-network supported by the Bavarian Ministry of Health.

Keywords: Puumala virus, bank vole, climate change, altitude

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## Distribution of mosquitoes and frequency of virus infection differ in disturbed and undisturbed rainforest areas

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It is widely accepted that the majority of emerging disease outbreaks occur in the tropics. At present, however, we lack both an understanding of pathogen prevalence in remote rainforest areas and reliable detection systems for novel pathogens. Monitoring of pathogen prevalence and circulation at the interface of pristine rainforests and disturbed landscapes is crucial for emerging disease surveillance and forecasting.

We present an approach to studying the variation in mosquito distribution and concomitant viral infections along anthropogenic disturbance gradients. In a pilot study we analyzed mosquito distribution along a transect of a West African rainforest edge area. Variation was observed in the abundance of Anopheles, Aedes, Culex and Uranotaenia mosquitoes between different habitat types. Screening of trapped mosquitoes led to the isolation of seven uncharacterized viruses of the families Bunyaviridae, Coronaviridae, Flaviviridae, Reoviridae and Rhabdoviridae. Specific screenings for these viruses in mosquitoes caught along the gradient indicated a trend toward infection with specific viruses in specific mosquito genera that differed by habitat. These data will be compared with data from further surveillance campaings with broader sampling approaches in Kibale National Park, Uganda and the Palenque National Park, Mexico.

Furthermore, experimental model systems to asses the impact of host- and geographic range alterations on viral pathogenicity will be discussed.

Keywords: Arboviruses, emerging infectious diseases, anthropogenic disturbance

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## Sandfly Fever Virus activity in Central/Northern Anatolia, Turkey: First report of Toscana virus infections

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Arthropod-borne sandfly fever viruses (SFV) cause febrile diseases as well as aseptic meningitis/encephalitis and include serotypes that comprise Sandfly fever Sicilian Virus (SFSV), Sandfly fever Naples Virus (SFNV) and Toscana Virus (TOSV). Infections are endemic in the Mediterranean basin and data on SFV activity in Turkey are limited. In this study, sera from 1533 blood donors from Ankara, Konya, Eskisehir and Zonguldak provinces of Turkey were evaluated for SFV exposure by indirect immunofluorescence test (IIFT) and confirmed by virus neutralization test (VNT). One hundred and two patients with central nervous system (CNS) infections of unknown etiology were also evaluated via IIFT and real-time reverse-transcription PCR for SFV/TOSV infections. Rate of overall IgG reactivity in IIFT was 32.9% (505/1533). TOSV exposure was confirmed by VNT in all study regions. Exposure to the recently-identified serotype Sandfly Turkish Virus, as evaluated by VNT, was revealed in Konya and Ankara. SFNV exposure was identified in Konya and SFSV was observed to be present in each region except Zonguldak. TOSV RNA was detected in 15.7% (16/102) and were accompanied by TOSV IgM in 25% (4/16) of the patients. Partial L- and S-segment sequences suggest that the Turkish TOSV sequences can be grouped to TOSV genotype A strains. Exposure to TOSV and other SFV serotypes were revealed in blood donors and CNS infections by TOSV were identified for the first time in Turkey. Infections are observed to be endemic in Central Anatolia and should be considered as etiologic agents in cases/outbreaks of fever of unknown origin and aseptic meningitis.

Keywords: Sandfly fever viruses, Toscana virus

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## Toscana virus-associated encephalitis is emerging north of the Alps

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Background Toscana virus (TOSV), a member of the bunyavirus family (genus Phlebovirus), is in up to 80% the major cause of viral meningitis acquired during the summer months in the Mediterranean countries. Until today only imported cases have been reported in central Europe, although the main vector of TOSV, Phlebotomus perniciosus, was primarily detected in 2001 in the southern parts of this geographical area.
Methods A number of 1773 hospitalized patients with suspected meningitis or encephalitis (ME) were grouped as non-infectious origin (n = 1374), infectious and parainfectious origin with known (n=249) and with unknown etiology (n=150). Serum samples were investigated by serology and CSF samples by real-time PCR or autochthonous IgG production to determine viral infection.
Based on serology and real-time PCR we discovered the first autochthonous human TOSV infections in the Upper Rhine valley proving the northward spread of TOSV across the alpine mountains, which separate southern Europe from central Europe. Up to 6.6% of cases diagnosed as ME of unknown etiology turned out to be TOSV infections. In the ME group of known etiology 26 % were caused by varicella zoster virus, 19% were tick borne encephalitis virus (TBEV) infections and 14% were due to Borrelia burgdorferi. In 11% of this group herpes simplex viruses type 1 or 2 were diagnosed, only 2% were due to enteroviruses and 28% of the cases included other viruses, bacterial or parasitic infections.
Interpretation Emerging TOSV has to be considered in diagnosis and management of infectious neurologic disease in the most southern parts of central Europe.

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# Session 2 – Pharmacology, Therapeutics and Resistance

## Selective attenuation of influenza A viruses by targeting the polymerase subunit assembly

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To develop novel attenuation strategies applicable for all influenza A viruses, we specifically targeted the highly conserved protein-protein interaction site between the viral polymerase subunits PA and PB1. We assumed that impaired binding between both subunits would result in decreased polymerase activity and, as a consequence, in reduced replication efficiency and viral growth. Here, we provide proof of concept that it is possible to generate such polymerase assembly mutants and further show that these mutant viruses are highly attenuated in mice. In addition, mice vaccinated with these polymerase assembly mutants were protected from lethal challenge with influenza A viruses. Importantly, the attenuation of these mutant viruses was maintained in mice lacking a functional type-I IFN$\alpha$ receptor, indicating that the attenuation is not dependent on the antiviral state of the host. In summary, we provide evidence that the specific impairment of polymerase subunit assembly represents a novel strategy for live vaccine development of influenza A viruses.

Keywords: Binding Affinity, Polymerase, Conservation, Vaccines, Attenuation

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## Oseltamivir-resistant variants of the 2009 pandemic H1N1 influenza A virus are not attenuated in the guinea pig and ferret transmission models

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Oseltamivir is routinely used worldwide for the treatment of severe influenza A infection, and should drug-resistant pandemic 2009 H1N1 viruses become widespread, this potent defence strategy might fail. Oseltamivir-resistant variants of the pandemic 2009 H1N1 influenza A virus have been detected in a substantial number of patients but, to date, the mutant viruses have not moved into circulation in the general population. It is not known whether the resistance mutations in the viral neuraminidase reduce viral fitness. We addressed this question by studying transmission of oseltamivir-resistant mutants derived from two different isolates of the pandemic H1N1 virus in both the guinea pig and ferret transmission models. In vitro, the virus readily acquired a single histidine to tyrosine mutation at position 275 (H275Y) in the viral neuraminidase when serially passaged in cell culture with increasing concentrations of oseltamivir. This mutation conferred a high degree of resistance to oseltamivir but not zanamivir. Unexpectedly, in guinea pigs and ferrets the fitness of the mutant virus was not impaired detectably and both wild-type and mutant viruses were transmitted equally well from animals that were initially inoculated with 1:1 virus mixtures to naïve contacts. Our data suggest that the currently circulating pandemic 2009 H1N1 virus has a high potential to acquire drug-resistance without losing fitness.

Keywords: Influenza, oseltamivir-resistance, neurami-nidase, viral fitness

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## Killing activity and protective efficacy of recombinant γ-phage lysin and synthetic theta defensins against *Bacillus anthracis*

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Concern regarding the development of antibiotic resistant strains of Bacillus anthracis has led to the investigation of alternative means of anthrax therapy. Moreover, antibiotic therapy alone does not prevent the fatal activity of the toxins. Here, the bactericidal effects of recombinant γ phage lysin (rPlyG), native α-defensins (gift of the Max-Planck-Institute of Infection Biology, Prof. S.H.E. Kaufmann, Berlin), and synthetic retrocyclins (Rcs), which are humanized θ-defensins, were tested against B. anthracis spores, germinated spores, and encapsulated bacilli in vitro. Their protective efficacy was tested in infection trials in mice. Our results show that (i) encapsulated bacilli were sensitive to all bactericidal agents, whereas spores were completely resistant; (ii) in vitro B. anthracis spores have to germinate for 90 min before they are fully susceptible to rPlyG, while the defensins can kill the bacilli even after 15 min of germination; (iii) defensins require 1-2 hrs for full bactericidal efficacy while rPlyG lyse the bacilli within 20-30 min; (iv) bactericidal efficacy of all agents is greatly reduced in blood serum compared with buffer solutions; (v) efficacy data from in vitro bactericidal tests do not predict the outcome of test for protectivity in vivo; (vi) rPlyg can protect NMRI mice after s. c. infection with Ames spores, but was not effective in treating mice infected i. v. with encapsulated bacilli; (vii) RCs protected A/J mice when given after s. c. challenge with Sterne spores, but was not effective in NMRI mice after infection with encapsulated cells of the Ames strains; (viii) RC treatment at 12h intervals appears to be better than 24h intervals potentially indicating a short half-life of RCs; (ix) combination treatment of rPlyG and RC in NMRI-mice, challenged with Ames spores showed increased protection compared to treatment with rPlyG only; (x) administration of 3x 50 mg rPlyG did not protect NZW rabbits from anthrax.

Keywords: lysin, defensin, anthrax

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## Approaches to the antimicrobial treatment of persistent *Chlamydia psittaci* infections

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Chlamydiae are pathogens which can persist in host cells. This persistence is related to chronic disease in that antibiotic therapy may fail. There is little knowledge of the antibiotic sensitivity of the zoonotic species Chlamydia psittaci (Cp).
We determined the minimal inhibitory concentration (MIC) of several antibiotics against an active Cp infection using the epithelial cell line A549. The MICs for macrolides, doxycycline and rifampicin ranged between 0.016 and 0.08 µg/ ml whereas those for quinolones were higher. To study the effects of antibiotics on persistent Cp an IFNgamma-mediated persistence model was established. After treatment with doxycycline, macrolides, and quinolones the number of recoverable Cp was higher than after treatment with rifampicin, indicating that a monotherapy is limited in eradicating persistent chlamydiae. Since persistent chlamydiae are non-cultivable, there are limitations to monitor antimicrobial effects by determination of recoverable infectious chlamydiae. Therefore, we established a real-time PCR assay using several chlamydial genes (e.g. ompA, groEL, ftsW) which showed different transcription patterns depending on the antibiotic added, the timepoint of addition, and the duration of treatment.
Our experiments revealed, that the efficacy of antibiotic monotherapy of persistent chlamydiae is limited. Real-time PCR assays can be used as a reliable tool to evaluate antibiotic combination for a successful eradication of persistent chlamydiae.

Keywords: Chlamydia psittaci, persistence, antimicrobial therapy

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## Extended-spectrum beta-lactamase producing Enterobacteriaceae in animals: what does the future hold for us?

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In the last decades, a dramatic increase of extended spectrum beta-lactamases producing (ESBL) Enterobacteriaceae as cause of significant community- and hospital-associated infections has occurred worldwide. Likewise, there are initial observations about the occurrence of such multiresistant strains in the veterinary field, although available data are still scanty compared with the situation in human medicine.
We therefore set up a study to unravel the presence of ESBL-producing isolates among Enterobacteriaceae spp., (E. coli, Citrobacter spp., Klebsiella spp., Enterobacter spp., Serratia spp., and Salmonella enterica ssp. enterica serovars) implicated in infections of different anatomical sites (urinary, respiratory, and intestinal tract, soft tissue, etc.). In a 22 months period about 20.000, mainly originating from cats, dogs, and horses strains, were consecutively isolated from clinical samples sent for diagnostic purposes.
Different rates of ESBL-producing strains were detected in single bacterial species, e.g. 2.8% in E. coli, 6.3% in K. pneumoniae ssp. pneumoniae, 9.2% in E. cloacae, and 26.5% in C. freundii. Considering the type of ESBL and the phylogenetic lineage of the respective strains, as determined by MLST for E. coli and Klebsiella spp., further analyses demonstrated that a number of ESBL-positive strains showed high similarities to emerging pathogenic clones observed in humans, thus strongly supporting the idea of transmission scenarios between animals and humans.

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# Session 3 – Wildlilfe Zoonoses

## Wildlife from Germany as hosts of multiresistant E. coli

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Wildlife animals are known to disseminate zoonotic bacteria of human and animal health concern and may likewise contribute to the dissemination of multiresistant zoonotic bacteria as well. Indeed the increasing prevalence of antimicrobial resistant bacteria in human patients and in livestock is accompanied by a co-emergence of multiresistant bacteria in wildlife species all over the globe. To assess this possible "wildlife hazard", it is important to determine how widely such bacteria have already spread into different ecosystems. Accordingly, our recent studies focussed on groups of wildlife of particular interest with respect to antimicrobial resistance: (i) wild birds as they most likely serve as long distance vectors of resistant bacteria and (ii) synanthropic rodents, which might require particular attention due to their close contact with humans and livestock. Along with these we do not only detect high rates of antimicrobial resistance in wildlife but we also recover multiresistant extended-spectrum beta-lactamase (ESBL) producing E. coli. As an example, the isolation of E. coli from wild birds revealed the occurrence of CTX-M-15-producing strains of multilocus sequence type ST648 which has also been detected in human clinical cases. E. coli isolates from urban rats exhibited a high prevalence of multiresistant isolates (25-30%) including the human pandemic B2-O25b:H4-ST131 ESBL E. coli clone. Our findings suggest that ESBL producing E. coli are commonly present in wildlife. To unravel the relevance of wildlife hosts as sources of ESBL-producing Enterobacteriaceae and to determine their contribution to the spread of antimicrobial resistant E. coli, a detailed surveillance of wildlife hosts should be implanted.

Keywords: Antibiotic resistance, E. coli, wildlife

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# The influence of the environment on *Trypanosoma cruzi* and other trypanosomes in Panamanian fruit bats

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We studied the trypanosome prevalence and species composition in two fruit bats species, Artibeus jamaicensis and A. lituratus, in Panama. More than 300 blood samples were taken and investigated by PCR and sequence analysis of a fragment of the trypanosome ssrRNA gene. The sequencing results were analysed with BLAST, and a Phylogenetic tree constructed.
32.4% of all bats were infected with trypanosomes, and we found more than 15 different ssrRNA gene fragment sequences, corresponding to several strains of trypanosomes. The most common trypanosomes were from the T cruzi cluster (Schizotrypanum), including the pathogenic species T. cruzi cruzi, and also T. cruzi marinkellei – the latter of which is restricted to bats. Other trypanosomes included several types of Trypanosoma rangeli and three sequences belonging to an hitherto unknown cluster of bat trypanosomes. The bat habitat quality influenced the trypanosome distribution pattern: Bats in fragmented areas had a higher trypanosome prevalence. Interestingly, the prevalence of T. cruzi was higher in bats from disturbed habitats: T. cruzi cruzi even tripled in prevalence compared to numbers from undisturbed forests. Other types of trypanosomes were not as significantly affected by the environment. Along with the much higher prevalence of T. cruzi, multiple infections were more common in disturbed habitats. The increase of T. cruzi prevalence – an extremely generalist pathogen – in degraded habitats might be due to an impoverished host diversity, which in turn facilitates infection and increases the infection rate (dilution effect). Another factor could be fragmentation-induced changes in the vegetation, which may favour the vectors of T. cruzi.!

Keywords: bats, trypanosomes, T. cruzi, habitat fragmentation

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## Strong and specific amplification of Coronavirus associated with parturition in a bat maternity roost

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Bats host mammalian Coronaviruses and other emerging viral pathogens. Mechanisms of virus maintenance and amplification are unclear. We examined a maternity colony of Myotis myotis along a contiguous space of time in a private house's attic in a suburban neighbourhood located in Rhineland-Palatinate, Germany. One novel Coronavirus, one novel Adenovirus, and six novel Astroviruses were detected in pooled bat fecal samples. The coronaviruses, but not astroviruses or adenoviruses were massively amplified following parturition, yielding a 100% detection rate in bat droppings and virus concentrations up to 1,000 fold higher than those detected prior to parturition. Bat maternity roosts should remain undisturbed to avoid zoonotic risks.

Keywords: Bats, coronavirus, astrovirus, adenovirus, zoonotic risk assessment, reservoir, ecology

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## Novel Adenoviruses in wild primates: High genetic diversity and evidence for zoonotic transmissions

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More than 50 human adenoviruses (AdV) causing conjunctivitis, keratoconjunctivitis, acute respiratory diseases or gastroenteritis have been described and classified into the species A to G. In addition, a plethora of AdV from captive non-human primates (NHP) have been described. Evidence has been frequently reported that AdV exchange genes through recombination and have the potential for zoonotic transmission. Here we report on the identification of novel AdV in wild primates. Samples were collected in Africa for over ten years. AdV DPOL, pTP and Hexon sequences were amplified with universal AdV-PCR utilising degenerate primers and long-distance PCR, and compared to known AdV. A plethora of novel AdV belonging to seven species was discovered in chimpanzees, gorillas and the prey of chimpanzees, the colobus monkey. In a chimpanzee, a species F AdV was detected which tightly clusters with colobus AdV, indicating a horizontal transmission from colobus to chimpanzees in recent past. Most importantly, AdV were detected in two chimpanzees which were 98% identical to the human HAdV-D15 and 99% identical to HAdV-F40. These chimpanzees had contact to humans and where obviously infected by human-to-chimpanzee transmission, shedding further light on the zoonotic potential of adenoviruses. Studies on the AdV infecting populations with close contacts to NHP or bush meat will show whether NHP-AdV transmit to humans and possibly recombine with human AdV giving rise to novel pathogens.

Keywords: Adenovirus, wildlife, ape, nonhuman primate, zoonosis

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## Transmission of retroviruses in primate predator – prey systems

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Simian retroviruses have been precursors of all human retroviral pathogens. However, little is known about the prevalence, co-infection rates and genetic diversity of major retroviruses - simian immunodeficiency viruses (SIV), simian T-cell lymphotrophic viruses type 1 (STLV-1) and simian foamy viruses (SFV) - in wild populations of non-human primates. Also systematic studies on the transmission of such viruses among wild primates and from wild primates to humans are missing.
Here we present a unique set of data based on the analyses of samples taken from wild red colobus monkeys as well as from their natural predators – chimpanzees - from a defined study area in West Africa. In addition we also present preliminary data on retroviruses transmitted to the local human population through hunting and butchering of both of those primate species.
Given that more than 34 million people annually are in contact with at least five million tons of blood, meat, and organs from wildlife (bushmeat) and that of this, approximately 12% originates from non-human primates, it is clear that the results obtained in this study only can only be considered the tip of a iceberg. Frequent zoonotic transmission of retroviruses may be considered a permanent risk for the emergence of new diseases.

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# Session 4 – Epidemiology, Modelling and Risk Assessment

## The story with the stork or Why are ecological relations regaining popularity in zoonoses research?

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Understanding the impact of the behaviour, living conditions and genetic parameters on human health is a central concern of modern health research. Epidemiology therefore studies the distribution of diseases, their preliminary stages, their consequences and the factors influencing their distribution. For this purpose, in zoonoses research, information on several populations has to be considered.

One aspect of comparing this information is the so-called ecological relations. Ecological relations are methods of statistical analysis, whereby aggregations of individuals of a population are used as unit of analysis. Common regional aggregation levels are administrative summarisations, for example counties. An evaluation of possible risk factors based on these administrative districts is frequently carried out by means of correlation analyses.

Even though such a calculation appears to make sense initially, a great number of examples show that this procedure may lead to severe misinterpretations, as an ecological correlation always leads to a pseudo-correlation, if a fundamental risk factor remains unconsidered.

In this presentation this "ecological fallacy" is explained on the basis of historical and recent examples. It appears that ecological relations are completely inapplicable to prove causal coherence in zoonoses research. Therefore, we should furthermore refrain from drawing conclusions from the correlation between the stork's occurrence and the birth rate that exists in Germany.

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Keywords: ecological correlation, infectious diseases, risk factors, confounding, "ecological fallacy"

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## Bayesian time-space analysis of *Echinococcus multilocularis*-infections in foxes

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Alveolar echinococcosis is a rare human disease that ends often lethal if left untreated. It is caused by the larval stage of the cestode parasite, Echinococcus multilocularis. In central Europe, the main definitive host of E. multilocularis is the red fox (Vulpes vulpes). To assess the risk for human infection, it is important to monitor the epidemiological situation of E. multilocularis in its definitive hosts in time and space. A total of 26,220 foxes that were hunted or found dead in Thuringia, Germany, between 1990 and 2009 were examined for infection with E. multilocularis. Data on these foxes were analyzed using a hierarchical Bayesian space-time model. The distribution of the model parameters and their variability was estimated on the basis of the sample size, the number of cases per spatial unit and time interval, and an adjacency matrix of the municipalities by using a Markov chain Monte Carlo simulation technique to assess the spatial and temporal changes in the distribution of the parasite. In the study area, the prevalence increased from 11.9% (95% confidence interval 9.9-14.0%) to a maximum of 42.0 % (39.1-44.1%) in 2005. While the infection was present in foxes only in the North-western parts of Thuringia in 1990, it had spread over the entire state by 2004. These results demand increased vigilance for human alveolar echinococcosis in Thuringia.

Keywords: Alveolar echinococcosis, Echinococcus multilocularis, fox, epidemiology, model

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## Phylogeny and migration of Tick-Borne Encephalitis Viruses in Southern Germany

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Tick-borne encephalitis virus (TBEV) can be distinguished in three subtypes. Within the particular subtypes virus strains exhibit little genetic variation. By sequencing seven complete TBEV genomes we identified a variable region in the NS2 gene. We tested the E gene and the NS2a gene for their use for phylogenetic characterization of TBEV strains.
The E genes and NS2a genes of TBEV strains from ticks of four different regions in southeastern Germany were sequenced. All strains belong to the Western subtype of TBEV. One strain, AS33, exhibited unique nucelotide and amino acid sequences in the E gene with two unique amino acid exchanges (E51D, T128I) among all known TBEV strains. Eight TBEV strains (HM1 to 8) were found about 15 km distant to the AS33 focus, but did not exhibit the described unique sequences in the E gene. From another TBE focus, some 40 km further east, sequences (BUL1-10) were recovered from ticks. In the fourth focus near the city of Passau one strain (FS-1) was recovered from ticks. The comparison of the E and NS2a genes to the available TBEV sequences from Central Europe shows that the strains of FS-1, BUL1-8 and HM1-10 are more related to Slovak strains than to TBEV strains from the Czech Republic. Our data imply that TBEV strains of southeastern Germany were introduced via Austria along the Danube River and its side arms. The Bavarian-Bohemian Wood seems to form a barrier for the distribution of those strains to the west.

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## Detection of a new chlamydial agent in birds

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Examination of clinical samples from several outbreaks of avian chlamydiosis left a number of unclear findings, where PCR testing was positive for the family Chlamydiaceae, but negative for Chlamydophila psittaci, the causative agent of classical psittacosis.
We will present the results from investigations involving DNA microarray assay, sequencing and cell culture that revealed the existence of a new, so far unclassified member of the genus Chlamydophila.
Greatly facilitated by the use of a recently developed DNA microarray test, this obligate intracellular bacterium was found in several avian hosts, i.e. chickens, ducks, pigeons, and psittacine birds, in three European countries, i.e. Germany, France and Italy. The data suggest that it resembles the known chlamydial species in terms of morphology, growth requirements and characteristics, as well as DNA sequence similarity.
So far, the agent has been predominantly encountered in symptomless birds. However, we recently found indications of a pathogenic role, as the new Chlamydophila spp. was identified in two outbreaks of respiratory disease in psittacine birds, where no other chlamydial, bacterial or viral pathogen was detected. Notably, we also reported an infection in duck flocks that coincided with cases of atypical pneumonia in slaughterhouse workers, which raises the question of a zoonotic potential.

Keywords: Chlamydiaceae, Aves, pathogenicity, zoonotic potential

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## Chlamydiaceae in sheep flocks in central Germany

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Ovine enzootic abortion caused by Chlamydophila (C.) abortus leads to significant economic losses, but also poses a zoonotic threat, particularly to pregnant women. As valid epidemiological data are lacking to assess the likelihood of animal-to-human transmission, the aim was to determine the prevalence of Chlamydiaceae spp. in sheep flocks in central Germany.
Fourty sheep flocks with at least 100 ewes were randomly chosen, without prior knowledge of their abortion status. Vaginal and rectal swabs from 11 animals per flock were taken after lambing, as well as paired blood samples from 29 animals. In addition, placentae and aborted fetuses were collected.
Swabs and tissue samples were tested by real-time PCR specific for the family Chlamydiaceae. Positive samples were further examined by C. abortus-specific real-time PCR, ArrayTube DNA microarray and partial sequencing. In case of positive PCR results, a second vaginal swab was used to isolate chlamydiae by cell culture. The serological status was determined using a commercial antibody ELISA kit (CHEKIT Chlamydia, IDEXX).
Of the 17 flocks completely examined so far, 13 tested positive by PCR and 16 by ELISA. At lambing, 32 % (70/220) of the sheep were found to shed chlamydiae, the most frequently found species being C. abortus (21% of all sheep), followed by C. psittaci.
In conclusion, chlamydiae seem to be widespread in sheep and the presence of species other than C. abortus has to be taken into account.

Keywords: Chlamydiae, sheep, epidemiology

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# Session 5 – Cellular Pathogenesis

## Characterization of evolved SARS-Coronavirus variants in a reservoir and dead-end host context

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The evolutionary pathway of the 2002/03 pandemic SARS-CoV remains unclear. Bats represent the likely reservoir of most mammalian CoVs. During putative host transition from animals to humans, SARS-CoV evolved a 29 nucleotide (nt) deletion within the open reading frame (orf) 8.
Passaging of SARS-CoV on Vero cells led to another 45 nt deletion within the orf7b, resulting in a virus that was more sensitive to interferon (IFN). To investigate the influence of those two deletions on virus propagation in the context of different reservoirs and specifically bats, we used a reverse genetics approach on a bat and primate cell culture system.
Three recombinant SARS-CoV (rSCV) variants were generated from an infectious cDNA clone: 29nt-rSCV (full orf8 and orf7b); rSCV (truncated orf8, full orf7b) and Δ7b-rSCV (both orf8 and orf7b truncated). All three viruses could be rescued and showed similar plaque phenotypes. Virus growth kinetics revealed that all virus strains replicated efficiently in both primate cells (MA-104, Vero) and human angiotensin converting enzyme-2 transgenic bat cells from Rousettus aegyptiacus and Rhinolophus landeri. Although IFN-competent MA-104 primate cells produced low titres for all rSCV, efficiency of virion production was higher than in bat cells. The Δ7b-rSCV virus generally grew better in all cell lines. Virus adaptations upon zoonotic transmission may therefore not have been a pre-requisite for successful replication in dead-end hosts.

Keywords: SARS-Coronavirus, bats, reverse genetics, host transition, viral evolution

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## Novel insights into the proteolytic activation of influenza virus and SARS-coronavirus

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Proteolysis of influenza virus hemagglutinin and SARS-coronavirus spike protein (SARS-S) by host cell proteases is essential for viral infectivity but the proteases responsible and, in case of SARS-S, the cleavage sites are not well defined. Previous studies showed that the type II transmembrane serine proteases (TTSPs) TMPRSS2, HAT and TMPRSS4 can activate influenza viruses in transfected cells. We found that endogenous TMPRSS2 and TMPRSS4 expression was largely responsible for trypsin-independent influenza virus activation in Caco-2 cells and TMPRSS2 expression was detected in type II pneumocytes, indicating that this protease could support viral spread in humans. A comparative analysis of the expression of influenza virus-activating TTSPs is ongoing, and might reveal that different TTSPs support viral spread in different compartments of the respiratory tract. Collectively, our observations suggest an important role of TTSPs in influenza virus activation and highlight these enzymes as potential therapeutic targets. The SARS-coronavirus is activated in host cell endosomes by cathepsins but the cleavage sites in SARS-S are not well defined. We found that R667, which was previously implicated in SARS-S proteolysis, was dispensable for SARS-S activation by trypsin and cathepsin L in a virus-virus fusion assay and we obtained evidence for an alternative cleavage site in SARS-S. In addition, we found that different host cell proteases activated SARS-S for virus-cell and cell-cell fusion and that under certain conditions cathepsin activity was dispensable for SARS-S-driven infectious cell entry. These

results demonstrate that inhibition of multiple proteases might be required to suppress SARS-CoV spread in the infected host.

Keywords: influenza hemagglutinin, SARS spike, protease, TMPRSS2, TMPRSS4

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## Crk adaptor protein expression is required for efficient replication of avian influenza A viruses and controls JNK mediated apoptotic responses

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The non-structural protein 1 (A/NS1) of influenza A viruses (IAV) harbors several src homology domain (SH)-binding motifs which are required for interaction with cellular proteins, such as the p85beta subunit of PI3-kinase. A SH3-binding motif (aa 212-217 [PPLPPK]) within A/NS1 has been shown to be essential for binding to the cellular adaptor proteins Crk/CrkL. Both regulate diverse pathways in the cell including activation of the MAP kinase JNK, that was previously shown by us to mediate antiviral responses. To elucidate Crk/CrkL functions in infected cells we knocked-down expression of Crk/CrkL by a siRNA approach. We could demonstrate that only those IAV that encode an A/NS1-protein harboring the intact SH3-binding motif PPLPPK are attenuated upon downregulation of CrkI/II or CrkL, but not of CrkII alone. The PPLPPK site-harboring candidate strains could be discriminated from other strains by a pronounced viral activation of the JNK-ATF2 signaling module that was even further boosted upon knock-down of CrkI/II. Interestingly, this enhanced JNK activation did not alter type-I IFN-expression, but rather resulted in increased levels of virus-induced cell death and Caspase-9 cleavage. Our results imply that binding-capacity of A/NS1 to Crk/CrkL has evolved in virus strains that over-induce the antiviral acting JNK-ATF2 signaling-module and helps to suppress the detrimental apoptosis promoting action of this pathway.

Keywords: A/NS1, CrkI/II and CrkL, JNK-ATF2, JNK-mediated apoptosis

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## The different functions of PI3K during influenza virus replication

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The Phosphatidylinositol-3-kinase (PI3K) is induced upon influenza A virus (IAV) infection by diverse mechanisms and at different time points during replication. Thereby the kinase fulfils several functions in the infected cells. While the PI3K was actually shown to induce antiviral activity via the activation of the interferon-regulatory factor 3, recent results further revealed virus supportive functions of the kinase. We could show that the very early transient activation of PI3K is observed during virus attachment and is required for efficient virus uptake. In this connection we identified receptor tyrosine kinases, such as the epidermal growth factor receptor as mediator of IAV induced PI3K activity. The later, more transient PI3K activation is dependent on the expression of the viral non-structural NS1 protein and seems to inhibit premature apoptosis. It was demonstrated by us and others that PI3K activation occurs upon direct interaction of the NS1 protein to the regulatory subunits of PI3K p85 alpha and beta. Several reports proposed that two src homology (SH)-binding motifs within NS1 (aa89 [YXXXM] and aa164-167 [PXXP]) may mediate binding to p85 beta. Our work confirmed this observation. However, mutant viruses of the NS1 (Y89F) only showed marginal differences to wild-type viruses with regard to their replication fitness. More detailed analysis suggested that besides expression of the NS1 there are alternative virus-induced mechanisms to activate PI3K. Here we demonstrate that this additional inducer is viral RNA (vRNA), which accumulates during infection.

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## NS reassortment of an H7-type HPAIV affects its propagation by altering the regulation of viral RNA production and anti-viral host response

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Highly pathogenic avian influenza viruses (HPAIV) with reassorted NS segments from H5- and H7-type avian virus strains placed in the genetic background of A/FPV/Rostock/34 HAPIV (H7N1) were generated by reverse genetics. Virological characterisations demonstrated that growth kinetics of the reassortant viruses differed from the wild type FPV and depended on cells of mammalian or avian origin. Surprisingly, molecular analysis revealed that the different reassortant NS segments were not only responsible for alterations in the anti-viral host response, but furthermore affected viral genome replication and transcription as well as nuclear RNP export. RNP reconstitution experiments demonstrated that the effects on accumulation of viral RNA species depended on the specific NS-segment as well as on the genetic background of the RdRp. IFN-beta expression and the induction of apoptosis were found to be inversely correlated with the magnitude of viral growth, while the NS allele, virus subtype and levels of NS1 protein expression showed no correlation. Thus, these results demonstrate that the origin of NS segment can have a dramatic effect on the replication efficiency and host range of HPAIV. Overall our data suggest that the propagation of NS reassortant influenza viruses is affected at multiple steps of the viral lifecycle as a result of the different activities of the NS1 protein on multiple viral and host functions.

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## Getting it out: Potential role of influenza a virus non-structural protein 1 (NS1) in viral mRNA Export

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Influenza viruses replicate and transcribe their genomic RNA in the nucleus of the host cell and therefore depend on nuclear host functions, such as mRNA splicing and export. In metazoans, transport of mRNA is tightly linked to splicing during which export factors are recruited to processed mRNAs. As most influenza virus mRNAs are not spliced, it is of interest to understand how viral mRNAs enter the export pathway and which factors are involved in this process. Recently, the NS1 protein that is known to antagonize the host interferon response was also shown to associate with viral mRNA, which led us to hypothesize that the NS1 protein promotes viral mRNA export.
We used a bimolecular fluorescence complementation (BiFC) assay that facilitates the detection of protein interactions in situ to screen for NS1 protein binding partners in the cellular mRNA export pathway. The BiFC screening revealed that the NS1 protein forms a complex with the mRNA export factors Aly and SF2/ASF. Both interactions were detected exclusively in the nucleoplasm of the cell. In addition, in vitro binding assays showed that purified NS1 protein binds directly to recombinant Aly and SF2/ASF, indicating a direct interaction in the host cell. Finally, we showed that viral mRNA was precipitated with NS1 and SF2/ASF. These findings suggest a model in which the NS1 protein acts as an adapter protein that links viral mRNA to the cellular export machinery by recruiting specialised host factors.

Keywords: NS1, mRNA export, BiFC, Aly, SF2/ASF

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# *Coxiella burnetii* type IV secretion substrate AnkG inhibits host cell apoptosis by modulating p32 activity

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*Coxiella burnetii*, the causative agent of human Q fever, is an obligate intracellular pathogen that encodes a type IV secretion system related to the *Legionella pneumophila* Dot/Icm system. It is predicted that proteins translocated by the type IV secretion system manipulate the host cell to permit bacterial survival and replication. We recently demonstrated that several different *C. burnetii* proteins with ankyrin repeat-containing domains could be delivered into the host cells by type IV secretion system. In agreement with the hypothesis that type IV secretion system substrates manipulate host cell pathway it was shown that one of the *C. burnetii* type IV substrate inhibit apoptosis. Introduction of AnkG into *L. pneumophila* results in intracellular growth of *L. pneumophila* in host cells that normally restrict replication by preventing the induction of apoptosis. AnkG binds specifically the mammalian protein p32 and interfered with the pro-apoptotic functions of p32. AnkG neutralized the ability of p32 to modulate RNA splicing of Bcl-x. Inhibition of p32 by AnkG resulted in reduced production of an alternatively-spliced transcript encoding the pro-apoptotic Bcl-$x_s$ protein. A similar defect in Bcl-x splicing occurred in cells infected with *C. burnetii*. Thus, modulation of p32 activity by AnkG represents a novel mechanism to inhibit host cell apoptosis.

Keywords: *Coxiella burnetii*; type IV secretions system; apoptosis; ankyrin repeats

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## Tracing host cell determinants in Chlamydia infection

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Chlamydiae are obligate intracellular pathogens that can cause both acute and chronic infections in humans and animals, ranging from pulmonary, ocular, urogenital and systemic infections. They modulate the host cell in order to generate optimal conditions for their growth. Definitive investigations are hampered by the fact that Chlamydiae cannot be grown extracellularly and by the lack of a gene transfer system to stably introduce DNA into these organisms. Hence, manipulation of the host is the primary strategy to explore Chlamydia host interactions. We have developed a global approach to study Chlamydia host cell interaction. The assay is running on an automated robotic platform. Infection is monitored in an immunofluorescent readout and quantified by automated microscopy and image analysis. We are performing large-scale siRNA screens (human genome-wide) and intermediate-scale, multiparameter screens using selected siRNA libraries with the human pathogens *Chlamydia trachomatis* and *Chlamydophila pneumoniae.* Comparable studies will be conducted with the zoonotic pathogen *Chlamydophila psittaci.* Using high-throughput RNA interference, we are tagging host cell determinants that are essential for bacterial entry, intracellular survival, replication and production of infectious progeny. Separate and comparative analyses of host cell - pathogen dependencies give us insight into the pathogenesis mechanisms of Chlamydia species and allow the identification of new drug targets for the treatment of Chlamydia infection.

Keywords: Chlamydia, genome-wide siRNA screen, drug targets

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## Hantavirus-induced kidney dysfunction is caused by disruption of tight junctions in renal endothelial and epithelial cells

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Hantaviruses are emerging rodent-borne pathogens that cause hemorrhagic fever in humans. The clinical picture is characterized by enhanced permeability. It is assumed that the infection of endothelial cells leads to capillary leakage. Old World hantaviruses infect mainly the kidney resulting in acute renal failure. The underlying molecular mechanisms of the pathogenesis of hantavirus-induced renal failure are not well characterized. Therefore, we investigated the pathomechanisms by analyzing renal biopsies of hantavirus-infected patients and by infection studies in human renal cell culture models.

We infected human primary renal cell types that are relevant for kidney function. Human tubular epithelial, glomerular endothelial cells and podocytes are susceptible to infection. The barrier function of monolayers depends on the integrity of cell-to-cell contacts mediated by tight junctions, specialized multiprotein complexes that control permeability. We observed that junctional proteins are mislocalized and decreased in infected tubular epithelial, glomerular endothelial cells and podocytes.

To confirm these results in hantavirus-infected patients, renal biopsies of patients with serologically confirmed hantavirus infection and control kidneys were analyzed by immunofluorescence. Hantaviral antigen was detected in glomerular and tubular cells. In infected patients tight junction proteins are reduced and redistributed from the cell contacts to the cytoplasm in comparison to uninfected patients.

The identification of target cells and characterization of viral-induced effects represent an important step in the understanding of capillary leakage induced by zoonotic infectious diseases.

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## Inhibition of IFN-β induction by Tribec virus

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Tribec virus (TRBV) is a tick - transmitted Orbivirus which is suspected to cause meningitis or encephalitis in humans. TBEV is prevalent in several European countries including Germany and may contribute to the high number of cases of aseptic meningitis/encephalitis of unknown etiology, however nothing is known about the pathogenic potential of TRBV.
The mammalian type I interferon (IFN) system is a first line of to defense to prevent viral infections. Many viruses have developed strategies to subvert this system. Since there is no data available concerning the interaction of TRBV with the type I IFN system, we explored the capability of TRBV to grow in type I IFN competent cells and its possible interference with IFN - β induction.
As demonstrated by non-invasive impedance measurement (xCELLigence system), TRBV is able to infect IFN competent murine and human cell lines. Furthermore, TRBV supported the growth of a type I IFN-sensitive virus (RVFV clone 13) in these cells. TRBV was also able to grow in human 293 cells without inducing IFN-β transcription. Likewise, in luciferase reporter assays TRBV infection did not lead to the activation of the IFN-β promoter. To elucidate whether this is an active inhibition of IFN-β induction, TRBV-infected cells were treated with VSV-RNA or infected with RVFV clone. While transfection of VSV-RNA or infection with RVFV clone 13 led to efficient activation of the IFN-β promoter in 293 cells, additional TRBVinfection abrogated this promoter activation indicating active inhibition by TRBV. Presence of TRBV did not impair RVFV clone 13 replication since we did not observe a decrease of RVFV clone 13 specific transcripts or a drop in production of infectious virus. At the same time however, a marked decrease of endogenous IFN - β transcripts was detected. Additionally, RVFV clone 13 induced transcription of ISG56 (which is mediated by activated IRF3) was also diminished in the presence of TRBV. Taken together, our data indicate that TRBV

actively inhibits IFN - β induction, a mechanism which was not reported for any other Orbivirus so far. Currently we are trying to identify the viral gene product and the underlying mechanism which are responsible for the type I IFN antagonism.

Keywords: Tribec virus, type I interferon system

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## Dissection of species barriers with inter-genotypic Lyssavirus chimeras

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Rabies virus (RABV) and related lyssaviruses cause lethal encephalitis in a wide range of mammals. Despite the broad host spectrum of lyssaviruses, host dependent peculiarities in virus replication, pathogenicitiy and in virus transmission, that might represent species barriers in terms of spill-over and manifestation in novel host populations, are suspected. In order to dissect the individual viral functions involved in host dependent virus replication, we transferred individual genes between genotypically distinct lyssaviruses that are either adapted to bats or to terrestrial mammals. Introduction of structural envelope proteins from bat associated European bat lyssavirus types 1 and 2 (EBLV-1/2; genotypes 5 and 6) into a classical rabies genetic backbone (RABV, genotype 1) showed, that inter-genotypic exchanges of the glyco- and matrix protein genes (G and M) are feasible. *In vitro* characterization of chimeras bearing the heterologous M from EBLV-1 strongly indicated different properties of EBLV-1 and RABV M in virus assembly, suggesting an M-dependent and genotype-specific function that could contribute to the host dependent replication potential of different lyssaviruses. Replacement of G led to viruses that replicated rather similar in cell culture. Since G is the only viral surface antigen and is referred as the major pathogenicity factor of lyssaviruses, G-chimeras are currently being investigated in mice with regard to G-dependent differences in virus pathogenicity.

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# Session 6 – Immunity and Pathogen-Host-Interaction

## Profiling human and animal antibody responses to zoonotic infections by *Chlamydia abortus* leads to detection of potential virulence factors

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The obligate intracellular bacterium C. abortus is the causative agent of enzootic abortion of ewes and can also be transmitted to humans. Identification of immunoreactive chlamydial proteins may lead to the detection of virulence factors, which become targets of the immune defence by interacting with host cell components. We screened C. abortus proteins in large scale by serological 2D proteomic analysis and by screening of a C. abortus specific expression gene bank in E. coli using sera of naturally and experimentally infected sheep as well as human sera from pregnant women with serious C. abortus infections. Finally about 60 chlamydial antigens were identified. While most of these proteins are involved in macromolecular processes and energy metabolism, the function of eight "hypothetical proteins" remains unclear and this study presents the first experimental evidence for their expression in vivo. Interestingly four of the "hypothetical proteins" are predicted to be secreted by the Type III secretion system and therefore represent potential virulence factors in chlamydial pathogenesis. Further characterization of these proteins by gene expression analysis on RNA and protein level during the developmental cycle as well as analysis of the subcellular localization by indirect immunofluorescence and transmission electron microscopy is under way.

Keywords: Chlamydia abortus, Immunoreactive proteins, virulence factor

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## Identification and characterization of a novel regulator STM0029 which contributes to Salmonella intracellular survival and resistance to antimicrobial peptides

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Introduction:
Antimicrobial peptides (AMPs) are crucial components of the host innate immunity to eliminate pathogens. In this study, we report the identification of a novel regulator STM0029 involved in management of *Salmonella* intracellular survival.
Methods:
We first performed in vitro killing assay in various murine macrophage cell lines and *peripheral blood mononuclear cells (PBMC) to determine intracellular survival ability between the wildtpy and* ΔSTM0029 strain. Furthermore, different types of AMPs were tested the sensitivity of ΔSTM0029 strain comparing to the wildtype. Finally, the microarray analysis was performed to find out which genes were regulated.
Results:
An orphan putative transcriptional regulator STM0029 is 149 amino acid long. *Comparing the wildtype and ΔSTM0029 mutant* in murine macrophages as well as PBMC *showed* differences of the viable intracellular bacteria were observed between the wildtype and ΔSTM0029 after 24 hours post-infection. Further, expression data revealed that Salmonella STM0029 gene is affected by pmrAB, but not phoPQ. *Since* ΔSTM0029 mutant was more susceptible after infection in macrophage cell lines and PBMC, we therefore tested the bactericidal activity of *α-defensin-1, LL-37, β-defensin-1/-2, lysozyme and polymyxin*. ΔSTM0029 strain showed similar sensitivity patterns as ΔpmrAB strain whereas the wildtype was relatively resistant against AMPs. Finally, numerous of murein/peptidoglycan (PG) genes were found to be up-regulated under the *Δ*STM0029 background, suggesting STM0029 is involved in the regulation of PG-related genes against intracellular receptors recognition.
Discussion:

We defined the function of *STM0029,* which is de-repressed by the *pmrAB* and further represses the expression of a set of PG-related genes to avoid being recognized by PAMP receptors as well as *in resistance to antimicrobial peptides* to establish the intracellular survival niche successfully. This finding gives us the new insight to identify the specific genes involved in pathogen survival issues as potential targets to control human infections.

Keywords: Antimicrobial peptides, Intracellular survival, Peptido-glycan, PmrAB two component system

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## Botulinum neurotoxin serotype D attacks neurons via two carbohydrate binding sites in a ganglioside dependent manner

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The extraordinary high toxicity of botulinum neurotoxins primarily results from their specific binding and uptake into neurons. At motorneurons, the seven botulinum neurotoxin serotypes A-G inhibit acetylcholine release leading to flaccid paralysis. Uptake of botulinum neurotoxins A, B, E, F and G requires a dual interaction with gangliosides and the synaptic vesicle proteins synaptotagmin or SV2, whereas little is known about the entry mechanisms of the serotypes C and D displaying the lowest amino acid sequence identity compared to the other five serotypes.
Here, we demonstrate that the neurotoxicity of botulinum neurotoxin serotype D depends on the presence of gangliosides by employing phrenic nerve hemidiaphragm preparations derived from mice expressing GM3, GM2, GM1 and GD1a or only GM3. High resolution crystal structures of its 50 kDa cell binding domain HC and in complex with sialic acid as well as biological analyses of single site botulinum neurotoxin mutants identified two carbohydrate binding sites. One site is located at a position previously identified in botulinum neurotoxins A, B, E, F and G but lacking the conserved SXWY motif. The other site coordinating one molecule sialic acid resembles the second ganglioside binding pocket of tetanus neurotoxin, named the sialic acid pocket.

Keywords: botulinum neurotoxin D, ganglioside binding site, KO mice, sialic acid cocrystal structure, HC-fragment

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## Protective *Toxplasma gondii*-specific T cell responses require the T cell-specific expression of protein kinase C-θ

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Protein kinase C (PKC)-θ is important for the activation of autoreactive T cells but is thought to be of minor importance for T cell responses in infectious diseases suggesting PKC-θ as target for the treatment of T cell-mediated autoimmune diseases. To explore the function of PKC-θ in a chronic persisting infection, in which T cells are crucial for pathogen control, we infected BALB/c PKC-$\theta^{-/-}$ and PKC-$\theta^{+/+}$ wildtype mice with *Toxoplasma gondii*. PKC-$\theta^{-/-}$ mice succumbed to necrotizing *Toxoplasma* encephalitis due to an insufficient parasite control up to day 40, whereas wildtype mice survived. The number of *T. gondii*-specific CD4 and CD8 T cells was significantly reduced in PKC-$\theta^{-/-}$ mice resulting in an impaired production of protective cytokines (interferon-γ, tumor necrosis factor) and anti-parasitic effector molecules (inducible nitric oxide, IGTP) in spleen and brain. In addition, Th2 cells were reduced in infected PKC-$\theta^{-/-}$ mice, paralleled by a diminished GATA3 expression of PKC-$\theta^{-/-}$ CD4 T cells and a reduced *T. gondii*-specific IgG production in serum and cerebrospinal fluid. Western blot analysis of splenic CD4 and CD8 T cells revealed an impaired activation of the NF-κB, AP1, and MAPK pathway in *T. gondii*-infected PKC-$\theta^{-/-}$ mice. Adoptive transfer of wildtype CD4 plus CD8 T cells significantly protected PKC-$\theta^{-/-}$ mice from death by increasing numbers of interferon-γ-producing *T. gondii*-specific CD4 and CD8 T cells illustrating a cell-autonomous, protective function of PKC-θ in T cells. These findings imply that PKC-θ inhibition drastically impairs *T. gondii*-specific T cells responses with fatal consequences for intracerebral parasite control and survival.

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## Inhibition of IFN-γ-mediated immune responses by *Toxoplasma gondii* in host macrophages can be reversed by histon deacetylase inhibitors

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*Toxoplasma gondii* is one of the most widespread eukaryotic parasites and lead to zoonotic diseases in humans and livestock. Its transmission cycle critically depends on the ability to induce long-lasting infections in immunocompetent intermediate hosts. Cell-mediated immunity including the production of IFN-γ is critical for control of the obligatory intracellular parasite. However, we and others have shown previously that *T. gondii* partially counteracts IFN-γ-mediated immunity. Here, we determined cellular mechanisms of this evasion strategy. Microarray analyses of the IFN-γ-regulated transcriptome of murine macrophages revealed that infection with *T. gondii* led to a general defect of infected macrophages to respond to IFN-γ. Analysis of the underlying mechanisms revealed a defect in the activity of STAT1-responsive promoters despite the activation and nuclear translocation of this transcription factor in response to IFN-γ. Chromatin immunoprecipitation showed that *T. gondii* inhibited the acetylation of histon H4, i.e. a major prerequisite for chromatin remodelling prior to transcription. We hypothesized that such defective histon acetylation could be due to increased activity of histone deacetylases (HDAC) in the presence of the parasite. Importantly, treatment with HDAC inhibitors led to a partial recovery of IFN-γ-induced gene expression in *T. gondii*-infected macrophages. In addition, such treatment abrogated the defective MHC class II expression in *T. gondii*-infected macrophages. The data indicate that *T. gondii* inhibits IFN-γ-regulated immune responses via interference with chromatin remodelling which can be reversed *in vitro* by HDAC inhibitors.

Keywords: *Toxoplasma gondii*, pathogen-host interaction, IFN-g, histon acetylation, immune evasion

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## The cellular receptor for West Nile Virus - Interaction with integrins?!

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The initial interaction of *West Nile* virus (WNV), a member of the *Flaviviridae* family, with host cells by binding to the cell surface receptor and the extent to which this interaction defines cell and/or host tropism are not well known. A previous study by Chu and Ng, J Biol Chem 2004, illustrated that the integrin αvβ3 served as the receptor for WNV entry into cells. By contrast, Medigeshi et al., J Virol 2008, using another strain and different approaches demonstrated that WNV entry is independent of integrin αvβ3.
In the present study, a cell culture model was established to clarify the potential role of the integrin as a receptor or co-receptor within a receptor complex for WNV binding and entry. Embryonic mouse fibroblasts lacking either the integrin subunits αv, β1 or β3, and wild type cells were isolated and infected individually with four different WNV strains to reveal possible differences in their binding abilities by terms of replication. Our infection experiments showed that (i) all WNV strains, used in this study, are able to replicate in the described cell lines, (ii) replication in β3-deficient cells was substantially lower compared to wild type cells, (iii) replication onset and efficiency showed considerate variations between cell lines and strains. These results strongly support the involvement of integrin αvβ3 in WNV binding and internalization into host cells. However, other receptor-associated pathways may also exist for WNV entry.

Keywords: WNV, integrins, receptor, binding

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## Replication and cytokine induction of pandemic H1N1 and H5N1 influenza A viruses in a human lung explant model

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Human influenza A viruses (IAV) and highly pathogenic avian IAV of the H5N1 subtype propagate efficiently in the human respiratory tract while low pathogenic avian IAV show little replication capability. We hypothesize that the host's innate immunity plays an important role in this restriction and that highly pathogenic influenza viruses have evolved mechanisms to evade the human immune system.
To elucidate the contribution of the innate immunity to the species barrier, we established a lung explant infection model from authentic human patient material. In this model, different human and animal IAV were characterized regarding replication, tropism and cytokine induction by plaque assay, immunohistochemistry and ELISA, respectively.
Seasonal H3N2 virus as well as H5N1 virus isolated from a fatal human case infected type II pneumocytes and replicated efficiently whereas a low pathogenic avian and a classical swine IAV of the H1N1 subtype showed only little growth. Pandemic H1N1-2009 virus replicated to a similar extent as a seasonal H1N1 strain and also caused weak chemokine and cytokine responses. In contrast, infection of human lung tissue with a H5N1 virus led to a pronounced induction of various chemokines and cytokines including IP-10, MIP-1beta and IFN-beta, which, remarkably, did not prevent its efficient replication.
Taken together this model provides an experimental approach with high clinical relevance to study IAV infection in the human lung.

Keywords: cytokine induction, innate immunity, human lung

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## Highly pathogenic influenza virus infection of the thymus interferes with T lymphocyte development

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Highly pathogenic avian influenza A viruses (HPAIV) cause severe disease in humans. Still the basis for their increased pathogenesis particularly with regards to the younger population remains unclear. Additionally, the recent pandemic H1N1v outbreak in 2009 demonstrated the urgent need for a better understanding about influenza virus infection. In the present study we demonstrated that HPAIV infection of mice led not only to lung destruction but also to functional damage of the thymus. Moreover, respiratory dendritic cells (RDCs) in the lung functioned as targets for HPAIV infection being able to transport infectious virus from the lung into the thymus. In addition the pandemic H1N1v influenza virus was able to infect RDCs without a proper transport to the thymus. Especially the strong interference of HPAIV with the immune system is devastating for the host and can lead to severe lymphopenia. In summary, from our data we conclude that highly pathogenic influenza viruses are able to reach the thymus via dendritic cells and to interfere with T lymphocyte development. Moreover, this exceptional mechanism might not only be found in influenza virus infection but also might be the reason of the increased immune evasion of some new emerging pathogens.

Keywords: H5N1 influenza A virus, pandemic H1N1v, DC, thymus, lymphopenia

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## Insufficient protection of vaccinated chickens against Egyptian escape mutants of highly pathogenic avian influenza H5N1

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Highly pathogenic avian influenza viruses (HPAIV) pose a severe threat to poultry industry worldwide. In addition, due to a certain zoonotic potential of these viruses human populations may be at risk as well. An HPAIV lineage of subtype H5N1 which originated in Southeast Asia in 1996/1997 has spread across Eurasia since 2003 and entered Africa in 2005. Despite intense attempts to eradicate the virus endemic status is now reported from poultry populations in Indonesia and Egypt. Endemic infection of poultry increases risks of sporadic human infections, the majority of which had a fatal outcome. Vaccination is an auxiliary tool to control HPAIV in poultry. Potency of commercial vaccines regarding antigenic drift variants arising during endemicity is under discussion.

We present data on potency testing of different inactivated commercial and experimental whole H5 virus vaccines to protect against two representative challenge viruses which are currently co-circulating in Egypt. All vaccines induced clinical protection against challenge with classic Egyptian strains. In contrast, when challenged with a variant, antigenically drifted strain, only chickens vaccinated with a homologous Egyptian clade 2.2.1 virus or an inactivated re-assorted H5N1 strain (Re-5, clade 2.3) were protected.

These date emphasis that the continuous monitoring of vaccine-driven evolution of HPAIV H5N1 is essential when applying vaccination as tool to reduce the amount of circulating virus within the poultry population and at the human-animal interface.

Keywords: Avian Infleunza, H5N1, escape mutants

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## Inhibition of the RIG-I dependent signalling pathway by the influenza B virus NS1 protein

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The retinoic acid inducible gene I product (RIG-I) functions as a cellular sensor detecting 5´-PPP-RNA during influenza virus infection and triggering the antiviral interferon response. Recently, it has been shown that activation of RIG-I requires sequential binding of RNA and unanchored polyubiquitin chains synthesized by the E3 ubiquitin ligase TRIM25. Additionally, TRIM25 facilitates ubiquitin conjugation to activate RIG-I. 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.
Here, we elucidated the molecular mechanism on how the highly divergent influenza B virus blocks the antiviral response. Using RNAi we showed that RIG-I is essential for influenza B virus induced IFN-β production. Reporter assays of transiently transfected cells revealed that B/NS1 inhibits the RIG-I induced IFN-β promoter activity. Although B/NS1 was found in immunoprecipitable complexes with RIG-I, we could not detect a direct interaction of the two proteins using GST-pulldown. Interestingly, we observed reduced ubiquitination of RIG-I in response to B/NS1 expression and an interaction of B/NS1 with TRIM25 in a bimolecular fluorescence complementation assay.
In conclusion, our data suggest that B/NS1 inhibits the RIG-I dependent signalling pathway by binding to TRIM25 acting upstream of RIG-I and thus executing an important pathogen-host interaction in the influenza virus replication cycle.

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

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# A polymorphism in the hemagglutinin of a highly pathogenic H5N1 Influenza virus determines organ tropism in mice

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Positive selection of hemagglutinin (HA) variants in H5N1-infected patients reflects ongoing adaptation processes that may eventually support spread within the human population. However, little is known about the consequences of these amino acid changes in HA. By analyzing a virus isolate *A/Thailand/KAN-1/2004 (KAN-1)* from an H5N1-infected patient, we identified a polymorphism at the receptor binding site of HA ($HA_{222K}$ and $HA_{222E}$). While 222K represents a characteristic avian-virus-like amino acid, 222E was suggested to be the result of a positive selection in the lung of this patient. Infection of mice with the mixture revealed replication of both virus populations in the lung, whereas only the $HA_{222K}$ variant was found in the brain. Studies in mice infected with recombinant KAN-1 $HA_{222E}$ or $HA_{222K}$ confirmed that each virus variant replicated efficiently in the lung, although significantly higher titers were observed for rKAN-1 $HA_{222E}$. At early time points after infection only rKAN-1 $HA_{222K}$ was detectable in the brain. Surprisingly, at later time points an increase in brain titers of rKAN-1 $HA_{222E}$-infected animals was observed. Sequence analysis revealed that in all cases a reversion to $HA_{222K}$ had occurred. Receptor binding properties of rKAN-1 $HA_{222E}$ showed overall reduced binding affinity for synthetic sialylglycopolymers except one tested analogue. Thus, positive selection of HA mutants in humans can increase replication efficiency in the lung and influence organ tropism.

Keywords: HA, H5N1, Organ tropism, Sialic acid, Positive selection

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# Session 7 – Infrastructures, Methods and Diagnostics

# MLVAplus.net: Online database and analysis Tool for bacterial typing with MLVA, SNP and other categorical data

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Background:
Molecular typing of bacteria is essential for epidemiological purposes such as investigating the spreading of specific genotypes or for outbreak detection. Recently, typing using multiple locus variable number of tandem repeat analysis (MLVA) and single nucleotide polymorphisms (SNP) gained increased interest, in particular, because these methods create reproducible and portable results. Several online-accessible databases containing MLVA- or SNP-data for clinical strains exist already. However, all of these databases are limited to data analysis of a single typing method. Therefore, it was our aim to design an expandable database application for multiple typing data of various pathogens.
Methods:
MLVAplus.net is a web-based application implemented in Java that can be accessed with any standard web browser.
Results:
The MLVAplus.net application allows users to create online-accessible databases for a combination of MLVA, SNP and other categorical data. Access rights for each database can be modified to grant read and write access to the public or to specific users only. The available analysis features include distance calculation based on different typing data as well as drawing and exporting of graphs based on UPGMA-, neighbor-joining, and minimum spanning tree algorithms.
Conclusion:
MLVAplus.net is a powerful tool for analysis of microbial strains based on different categorical typing data. The application is freely accessible at http://www.mlvaplus.net.

Keywords: MLVA, SNP, database, online

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## A comprehensive next generation sequencing strategy for full-length genomes of influenza A

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The sudden and unexpected emergence and fast spread of the pandemic H1N1 swine origin Influenza A virus in 2009 has once more emphasized the need for comprehensive and quick diagnostic tools in this field. In order to meet this demand, we designed an all-embracing reverse transcription-PCR (RT-PCR) for generation of DNA from Influenza A virus genome segments for full-length genome sequencing on a Genome Sequencer FLX. The RT-PCRs are designed such that the risk of contamination of diagnostic real-time PCRs by sequencing amplicons is minimized. With the presented protocol we were able to generate virtually all amplicons (99.3 % success rate) from isolates representing all so far known 16 hemagglutinin and 9 neuraminidase subtypes. In addition, we successfully generated all amplicons from a recent pandemic Influenza A H1N1 virus. Three isolates were sequenced to analyse the suitability of the DNA for sequencing and high quality nearly full-length sequences could be established from the raw data in a de novo assembly.

Keywords: Influenza A, next generation sequencing, full-length genome

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## Peptide microarray-based serologic strain-typing of *Toxoplasma gondii* in infected humans from Germany

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*Toxoplasma gondii* isolates from Europe belong to clonal lineages I, II and III. Different genotypes might be associated with different clinical manifestations in humans and animals. To identify the clonal types of *T. gondii* by which patients and other infected subjects are infected and to study putative relationships between clonal types and clinical manifestations of *T. gondii*-infections we established a peptide-microarray test using synthetic peptides, which were derived from polymorphic, type-specific sites of 14 *T. gondii*-immunogens (Kong et al., 2003, J Inf Dis 187:1484–95). In our study we analysed 166 sera from serologically positive patients and subjects. We found that 57% of the sera predominantly reacted with type II, 5% with type I and 4% with type III specific peptides. In 12% of the sera type II/III, or type I/III, or type I/II-specific reactions were detected. 21% of the sera showed no reactions with type-specific peptides. Our results indicate that type II-specific reactions were overrepresented in tested sera from Germany. In addition we observed patients with type I- or type III-specific reactions. These results are in accord with genotyping results on *T. gondii*-oocysts in faeces from cats in Germany (Herrmann et al., 2010, Int J Parasitol 1;40:285-92). This is the first report on *T. gondii* serotyping from Germany by peptide-microarray analysis.

This project was funded by the Bundesministerium für Bildung und Forschung (grant 01 KI 0765) as part of the Toxonet01 Network (National Research Platform for Zoonoses).

Keywords: *Toxoplasma gondii*, peptide-microarray, serotyping, humans, synthetic peptides

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## A single mixed *Toxoplasma gondii* oocyst sample from Germany consists of many *T. gondii* clones of different virulences, genetically distinct from the clonal types I, II and III

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*Toxoplasma gondii* is a wide-spread parasite zoonosis that infects almost all vertebrates, including humans, world-wide. Wild and domestic felids act as definite hosts that shed the oocyst stage which becomes infectious after sporulation. It is characterised by a high resistance to environmental influences. *T. gondii* has a clonal population structure. In North America and Europe three clonal lineages, named Type I, II and III, are predominately found. Infection with a single Type I-parasite is sufficient to kill mice, whereas $10^3$ parasites of Type II or Type III are needed to achieve the same effect in mice. Sexual recombination between different *T. gondii* clonal types can only occur in felids. In our study, we identified a single oocyst sample from a naturally infected cat in Germany displaying a combination of Type II- and Type III-alleles at different loci (Herrmann et al.,2010.Int.J.Parasit.40:285-92). IFN-γ-knockout mice were infected with this oocyst sample. Parasites isolated from mouse tissues were subsequently cultivated in VERO cells. Individual atypical clones were identified by limiting dilution and shown to possess different virulence phenotypes in mice. The majority of *T. gondii* clones of this isolate were highly virulent in mice. However, also less virulent and avirulent clones could be identified. For the fist time, we could show that sexual recombination in felids happened in naturally infected cats and that new genotypes of high virulence can form by sexual recombination in cats under natural conditions.

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Keywords: *Toxoplasma gondii*, genotyping, virulence

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## Novel murine models to study *Campylobacter jejuni* infection

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Despite the prevalence of *Campylobacter* infections and the importance of the associated intestinal and systemic diseases worldwide, it is still unclear by which mechanisms *C. jejuni* causes inflammation. Detailed insights into immunomodulation and bacteria-host-interactions are restricted by the lack of adequate experimental vertebrate models. This is in particular due to the physiological colonization resistance of conventional mice which clear the pathogen within two days post infection. To overcome this limitation, we have modified murine models of intestinal inflammation thereby providing novel experimental tools to study the role of gut microbiota and innate responses in Campylobacter infection. The results demonstrate that the colonization resistance displayed against *C. jejuni* by conventional mice is caused by the distinct gut flora composition. Gnotobiotic mice generated by quintuple antibiotic treatment, mice recolonized with a human gut flora and young mice infected right after weaning were effectively colonized by *C. jejuni*. Moreover, *C. jejuni* readily colonized in mice with acute or chronic intestinal inflammation (ileitis or colitis). During acute ileitis the pathogen translocated into mesenteric lymph nodes, spleen, liver and blood. The analysis of immune responses in the colon of gnotobiotic mice and mice with a human microbiota *in situ* revealed that *C. jejuni* infection recruites neutrophils, B- and T cells, as well as regulatory T-cells to inflamed intestinal sites. Investigations of mice lacking Toll-like receptors 2, 4, 9 or general innate signalling pathways showed that *C. jejuni* LPS is essentially involved in initiatiation and exaggeration of inflammatory responses via MyD88-dependent signalling pathways.
We conclude that modification of the gut flora composition is a valuable measure to establish novel murine models for the discovery of colonization factors and immunomodulatory mechanisms of *C. jejuni*. The fact that the gut flora composition predisposes a host for infection with a gastrointestinal pathogen

might have preventive implications in particular for patients with chronic inflammatory bowel diseases.

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## Experimental poxvirus infection in common marmosets (*Callithrix jacchus*): a new primate model for Orthopox virus infections

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The increased number of zoonotic orthopoxvirus (OPV) infections as well as their potential use in bioterrorism is of concern as they can induce severe diseases in humans. New vaccines and therapeutics against OPV infections are urgently needed and have to be tested in primate models. A big drawback of existing OPV non-human primate (NHP) models is the inherent moderate to high virulence for humans of the used Monkeypox- and Variola virus strains. The objective of this study was to characterize the pathology of a new orthopoxvirus model in nonhuman primates and to compare it with smallpox infection. The virus was isolated during a lethal atypical epizootic orthopoxvirus infection in a colony of 80 New World monkeys in Lower Saxony, Germany. Sequencing and molecular characterization identified the virus as a new cowpox virus named calpox virus.

Infection experiments were performed with 28 common marmosets, which were exposed to varying doses of calpox virus by intravenous, oropharyngeal and intranasal application. Intranasaly, the median infectious dose corresponded to $8.3x10^2$ pfu of calpox virus. Infected animals developed OPV-symptoms and died within 4 - 15 days post infection. Histology revealed characteristic pox-like lesions in the affected organs.

Calpox virus disease progression and pathological findings in the common marmoset appears to be consistent with lethal OPV infections in humans and in other NHP models. Intranasal virus inoculation with low virus doses mimics the natural route of the human variola virus infection. Thus, the calpox virus/marmoset model can be considered as a suitable, cost efficient and relevant NHP model to investigate the mechanisms of orthopoxvirus pathogenesis and pathology and to evaluate new vaccines as well as antiviral therapies.

Keywords: Cowpox virus, calpox virus, Callithrix jacchus, New World monkey, nonhuman primate model

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# Poster Presentations

# Poster Session – Cellular Pathogenesis

**Number C1**

## Mycobacterium avium spp. modulate maturation of murine dendritic cells)

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*Mycobacterium avium ssp. paratuberculosis* (MAP) is the etiological agent of Johne's disease, a chronic, non-treatable granulomatous enteritis of ruminants. Since long MAP is discussed as an aetiological agent of Crohn´s disease in humans. MAP is genetically closely related to other subspecies of *Mycobacterium avium* which can cause mycobacteriosis in animals and immunocompromized humans. The mechanisms contributing to MAP tissue tropism and progression of inflammation in the gut, as well as its interaction with dendritic cells (DC) are still unclear. In this study we investigated the interaction of MAP and *Mycobacterium avium ssp. avium* (MAA) with murine bone marrow derived DC.

By transmission electron microscopy we were able to demonstrate that MAP was located in the DC within membrane surrounded vacuoles. By a bacterial survival assay we could show that MAP was able to survive in DC for more than 3 days.

Infections of DC with both viable and heat-killed MAP or MAA induced DC maturation which differed to the maturation induced by LPS. Maturation was accompanied by higher production of IL-10 and lower production of IL-12. Treatment of DC with mycobacterial lipoarabinomannan and lipomannan failed to induce IL-12 and IL-10, indicating that other heat-stable mycobacterial components appear to be responsible for activation of DC. Altogether, our results indicate that MAP and MAA are able to subvert DC function directly by infecting the DC.

Keywords: *Mycobacterium avium subspecies paratuberculosis*, Johne's disease, IL-10, IL-12, pathogenic mycobacteria

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**Number C2**

## Avian influenza viruses use different receptors for infection of the respiratory epithelium in different avian host species

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Influenza viruses initiate infection by binding of the hemagglutinin to cell surface receptors containing sialic acid as a crucial determinant. The distribution of α-2,3 and α-2,6-linked sialic acids on different cell types and the viral preference for these receptors is believed to determine both the cell and species specificity of the virus.
We have characterized the infection by avian influenza virus strains of the H7 and H9 subtypes in tracheal organ cultures (TOCs) from chicken and turkey. To analyze the role of sialic acids in the onset of infection, TOCs were pretreated with neuraminidase to protect the cells from virus infection. As expected, enzymatic pretreatment retarded the ciliostatic effect of the H7 subtype virus. By contrast, no protective effect on the ciliostasis was observed in chicken TOCs infected by an H9N2 strain. However, infection of turkey TOCs by the H9N2 virus was neuraminidase-sensitive. This infection pattern was confirmed by immunostaining.
Fluorescent staining using specific lectins to visualize α-2,3 and α-2,6-linked sialic acids on the cell surface revealed that both chicken and turkey respiratory epithelial cells contain α-2,3-linked sialic acids; however, α-2,6-linked sialic acids were found only on the surface of the turkey but not chicken respiratory epithelium.
These findings suggest that avian influenza viruses use different receptors on their host cells depending on both the subtype of the hemagglutinin and the host species.

Keywords: influenza, hemagglutinin, tracheal organ cultures, silic acids

**Number C3**

## Comparative analyses of virulence associated genes in LEE positive Shiga-toxin producing E. coli (STEC) and atypical enteropathogenic E. coli (aEPEC) from cattle

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Cattle are known to be a reservoir for human infections with Shiga- Toxin producing *E. coli* (STEC) and aEPEC. As aEPEC have been shown to share similarities with STEC, we compared the similarity of these two pathotypes in freshly isolated bovine *E. coli* from fecal samples of 1.000 slaughtered cattle. The isolates were analyzed for Locus of Enterocyte Effacement (LEE) -positive STEC and aEPEC by screening the genes *eae* and *escV*, Bundle forming pili (*bfp*), Shiga Toxin *(stx*) and concentrating on the Genomic island OI 122 which is known to be associated with virulence.
We identified 52 LEE positive STEC (5.2%), 72 aEPEC (7.2%) and 5 animals shed both pathogens. Our analysis revealed that 8 aEPEC harboured a complete (*pagC, sen, efa1/lifA*, *nleB* and *ent*) OI 122 (10.8%), 13 an incomplete OI 122 (19.4%) and 51 were OI 122 negative. None of the LEE-positive STEC had a complete OI 122, but 3 strains harboured an incomplete OI 122. 49 of the LEE-positive STEC isolates were OI 122 negative (94.2%).
For detailed insights into the phylogenetic relatedness of the OI 122 isolated strains, Multi locus sequence typing (MLST) was performed. Furthermore all aEPEC and LEE-positive STEC strains were tested for virulence-associated genes, like genes involved in iron acquisition, encoding toxins and adhesins. The genes *ehxA, paa, cdt, nleA, traT* and *iroN* were significantly associated with aEPEC in contrast to
LEE-positive STEC which were significantly associated with *nleB, ompA, mat, feoB* and *chuA*.
These data argue against a general relationship between aEPEC and LEE-positive STEC strains from bovines, as most of the strains differ regarding their possession of virulence-associated genes. However, as some strains share identical Sequence types

(STs), they might have evolved from common ancestors. Future work should address the question about what role the differences between aEPEC with complete OI-122 and aEPEC without complete OI-122 play in the emergence of pathogenic *E. coli*.

**Number C4**

## Identification of a dense granule protein GRA9 in Neospora caninum

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The intracellular parasite *Neospora caninum* belongs to the phylum apicomplexa. The definitive host of this parasite is the dog and cattle display the main intermediate hosts. This work deals with a comparison of the near related apicomplexan parasites *N. caninum* and *Toxoplasma gondii* regarding dense granule proteins which play important roles in host-parasite-relationships. Previously, we identified the dense granule protein GRA9 in T. gondii. Hence we want to describe a putative homologue in N. caninum. We analysed the protein sequence of *T. gondii* and *N. caninum* GRA9 by BLAST analysis. It became clear that the proteins reveal a sequence identity of 60%. Furthermore, we observed that GRA1 from both parasites also show a high sequence identity of 53%, whereas GRA2 and GRA3 show lower sequence identities of about 38% and 31%, respectively. By confocal microscopy, we showed that the *T. gondii* GRA9- and GRA1-specific antibodies detect the putative homologue proteins in *N. caninum* in which they were mainly located inside the parasite, whereas in *T. gondii* these proteins are mainly found within the parasitophorous vacuole (PV). Furthermore, we are interested in the targeting of *Nc*GRA9 within *T. gondii*. Therefore, a NcGRA9-HA construct was transfected into *T. gond*i. It became clear that the endogenous *Tg*GRA9 and the transfected *Nc*GRA9 show the same localization inside the parasite and within the PV. In future, GRA9-secretion and localization in *N. caninum* will be further specified.

Keywords: *Neospora caninum, Toxoplasma gondii*, GRA9

**Number C5**

## Immunoglobulin-binding protein EibG of shiga toxin-producing *Escherichia coli*: Different phenotypes and adhesion to human and bovine intestinal epithelial cells

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Shiga toxin (Stx) producing *Escherichia coli* (STEC) belong to a pathogen group of major interest because STEC-strains are able to cause severe disease in humans. Several STEC strains express a group of immunoglobulin-binding proteins: EibA, C, D, E, F and G, occurring separately or combined within a single strain. These proteins bind by nonimmune manner to immunoglobulins and enable bacteria to evade host immune response. Additionally, EibG is involved in bacterial adherence to host intestinal epithelial cells and *eibG*-positive strains show chain-like adherence pattern (CLAP). In this study, structural variation of *eibG*-allels was discovered by PCR within a large collection of STEC strains characterised by multilocus sequence typing (MLST). Analysis of 440 strains comprising 116 serotypes showed 37 *eae*-negative *eibG*-positive strains with 21 different *eibG*-allels allocate in three subtypes. Different CLAP-phenotypes in adhesion assays with human and bovine epithelial cells and their dependence on different subtypes were confirmed by *eibG*-expression in alternative genetic background *E. coli*. The subtypes were further characterized by MALDI-TOF-MS peptide mapping. Binding of EibG was found to be independent of glycosylation of the CH2-domain of the Fc-portion of IgG. Our results indicate EibG as a potential non-fimbrial adhesine which binding capacity to human and non-human epithelial cells and Ig-subtypes of various mammalian species requires further investigation.

Keywords: STEC, host pathogen interaction, allelic variation, MLST

**Number C6**

## Analysis of well-differentiated porcine airway epithelial cells as target cells for influenza viruses

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Swine are considered to serve as "mixing vessels" for the reassortment of genetic material from human, porcine, and avian influenza viruses. Primary host cells of influenza viruses are epithelial cells of the respiratory tract. To analyze the infection of the target cells of influenza viruses,we have established precision-cut lung slices (PCLS) as a culture system for well-differentiated respiratory epithelial cells. PCLS contain the differentiated epithelial cells (ciliated cells and mucus-producing cells) in the original setting. Culture systems for such cells have been described and used to analyze infection by human and avian influenza viruses. Comparable studies with porcine airway epithelial cells have not been reported. PCLS were stained for the presence of sialic acids using lectins from *Maackia amurensis* (MAA) and *Sambucus nigra* (SNA). Anti-nucleoprotein antibody were used for detection of infection by swine influenza viruses (H3N2) and avian influenza viruses (H9N2 and H7N7). The ciliated epithelial cells were found to express $\alpha$2,6-linked sialic acid more prominently than $\alpha$2,3-linked sialic acid. Staining of PCLS for viral antigen (NP) indicated that the epithelial cells were sensitive to infection by both porcine and avian influenza viruses. In future studies we will compare the efficiency of infection and analyze the adaptation process by performing serial passages of avian influenza virus in porcine PCLS.

**Number C7**

## Analysis of the sialic acid binding properties of influenza virus hemagglutinins

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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. 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 binding sites of influenza HA.
We have generated soluble HAs that can be used as lectins for detection of those sialoglycoconjugates that are potential interaction partners for influenza viruses. Fusion of the ectodomain to the Fc-component of a human IgG and to a modified GCN4 leucin zipper motif as trimerization domain results in chimeric proteins that are secreted into cell culture supernatant after transfection and were purified by FPLC.
Immunofluorescence binding tests showed a clear binding of the soluble HAs to the cell surface of MDCKII cells which is sensitive to pretreatment with sialidase, showing that the binding is sialic acid-dependent. This effect was quantified by flow cytometry. Binding of the soluble HAs was also detected to the epithelium of the respiratory tract from different species.
Future studies will further evaluate the potential of soluble HAs as a tool to investigate the receptor binding of Influenza A viruses.

Keywords: Influenza, soluble hemagglutinin, sialic acids, binding

**Number C8**

## Determination of a potential *Toxoplasma gondii* GRA9 protein complex

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*Toxoplasma gondii* is one of the most successful protozoan parasites infecting all warm-blooded animals, including humans. The parasite develops within a parasitophorous vacuole (PV). Major components of this PV are dense granule proteins (GRA proteins) which were secreted as soluble moieties and target to specific subcomponents within this PV. Our investigations focus on the 41 kDa protein GRA9, which stably associates with the network of membranous tubules and the PV membrane (PVM). To closer explore the function of GRA9 we analysed its secretion- and interaction-behaviour in relation to other GRA proteins. We show here that GRA9 co-localize with the dense granules proteins GRA1, GRA2, GRA3, GRA5 and GRA7. Co-immunoprecipitation-analysis demonstrates a direct interaction between GRA9 and GRA1 (the vacuolar lumen protein) and the PVM-associated proteins GRA3 and GRA5. Further we found out that the identified GRA9-interaction partners GRA1, GRA3 and GRA5 bind to each other as well, which indicates the existence of a potential GRA-protein complex. However, using a GRA5-deficient mutant we were able to verify that the absence of GRA5 does not influence the behaviour and distribution of GRA9. To explore GRA9 secretion and interaction in more detail, we exogenously expressed HA-tagged GRA9 as well as C-terminal truncated versions of GRA9 in RH tachyzoites. First results indicate that the C-terminus of GRA9 is essential and sufficient for the correct GRA9 secretion into the PV.

This project was funded by the Bundesministerium für Bildung und Forschung as part of the Toxonet01 Network (National Research Platform for Zoonoses).

Keywords: Toxoplasma gondii, GRA9

**Number C9**

## Cocrystal structure of Botulinum neurotoxin type c reveal that cell entry is dependent upon interaction with two ganglioside molecules

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The seven botulinum neurotoxin (BoNT) serotypes cause muscle paralysis by selectively cleaving core components of the vesicular fusion machinery inside motoneurons. Their extraordinary activity relies on the highly specific uptake by neuronal cells. Initially they bind to complex gangliosides via a conserved binding pocket which has so far been characterized in detail only for serotypes BoNT/A and B. An additional interaction with a protein receptor is required for neurotoxin uptake as shown for BoNT/A, B, E, F and G. The BoNT/B and G protein receptor binding site is located in the neighborhood of the ganglioside binding pocket at the tip of their cell binding domain H C . Information about corresponding sites is lacking for BoNT/C and D. Here, we report the identification and characterization of two new ganglioside binding pockets of BoNT/C based on high resolution cocrystal structures of BoNT/C H C in complex with sialyllactose or sialic acid. Substitution of Y1179 and L1203 forming a small pocket displaying hydrophobic interaction with sialic acid drastically decreased binding to neuronal membranes as well as toxicity at mice phrenic nerve preparations.
MALDI-TOF MS analysis of H C C wild type incubated with ganglioside GT1b proved the binding of two molecules GT1b. Proteinase K pretreatment of functional synaptosomes reduced the uptake of BoNT/A but not BoNT/C as demonstrated by immochemical detection of their cleaved neuronal protein substrate SNAP-25. Thus, this study delineates a variant double receptor scenario involving two glycolipid receptors.

Keywords: botulinum neurotoxin C, ganglioside binding site, KO mice, cocrystal structure, H C -fragment

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**Number C10**

## Analysis of defence mechanisms against *Toxoplasma gondii* triggered by IFN-γ in skeletal muscle cells *in vitro*

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Skeletal muscle cells are important for persistance of the obligatory intracellular *Toxoplasma gondii* and its transmission to humans via undercooked meat. IFN-γ plays a critical role in the cellular defence against *T. gondii*. In our experiments, we show that this is the case in murine skeletal muscle cells (SMC) as well. Addition of IFN-γ to SMC led to diminished infection- and replication rates of the parasite. Cell death assays showed that IFN-γ had no cytotoxic effect on the host cells due to an egress of the parasite.
Reactive NO species produced by iNOS have been previously described to be involved in defence against intracellular pathogens. Addition of IFN-γ to SMC led to an increase of nitrite concentration, i.e. a stable derivative of NO, which was, however, smaller in *Toxoplasma*-infected muscle cells as compared to non-infected controls. Anyhow, a significant increase of the parasite replication rates after the addition of the iNOS-inhibitor L-NIL to IFN-γ-activated SMC was not detected. Thus, an involvement of iNOS in the cellular defence against *T. gondii* was not found.
Furthermore, we show that the p47 GTPase Irga6 was localizing to the parasitophorous vacuoles in IFN-γ stimulated SMC, possibly leading to the disruption of the PV. Quantitative RT-PCR experiments confirmed that the p47 GTPase genes *Irga6* and *IGTP* as well as *NOS2* coding for iNOS were upregulated upon stimulation of SMC with IFN-γ. However, IFN-γ induced up-regulation of *NOS2*, *Irga6* and *IGTP* was partially diminished by concomitant infection with *T. gondii*.

Keywords: Toxoplasma, IFN- γ, skeletal muscle, immune defence

**Number C11**

## Host-specific differences in recognition of *Salmonella* serovars and bacterial molecular patterns

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The host immune response plays a decisive role in the ability for *Salmonella* serovars to establish infections in human and/or animal hosts, indicating serovar-specific interactions with the host plays a key role in determining the zoonotic potential of a given *Salmonella* serovar.
We have been studying host innate immune responses to infection by different *Salmonella* serovars and mutants using NFκB activation as a model for innate immune recognition of bacterial pathogens. We are using assays based on NFκB-dependent reporter constructs integrated into the genomes of cell lines of different species and qRT-PCR determinations of cytokine expression. Differences in the activation of NFκB in response to infection by different serovars as well as flagellar and muropeptide metabolism mutants of serovar Typhimurium (substrates for Toll-like receptor 5 and/or IPAF and NOD proteins, resp.) were observed in different host species, indicating different innate immune recognition of bacterial components. The results also showed that host species-dependent responses to infection were partially dependent on the cell type, either intestinal epithelia or macrophage, suggesting basic differences in host innate immune responses to gastrointestinal pathogens such as *Salmonella*.
The results will be discussed with regard to possible roles in host adaptation by *Salmonella* serovars and different pathogenesis of infections in animal species compared to human infections.

Keywords: *Salmonella*, host-adaptation, innate immunity

# Poster Session – Epidemiology, Modelling and Risk Assessment

**Number E1**

## A longitudinal study of two TBE foci in southern Germany: fluctuations in TBEV prevalence in ticks and risk assessment

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Tick-borne encephalitis (TBE) is the most important arbovirus disease in Central Europe and in Central Europe is mainly transmitted by the tick *Ixodes ricinus*. The incidence of human disease is correlated to tick activity. However, longitudinal studies on the activity of endemic foci are missing.
We present the results of a longitudinal study in two TBEV endemic foci in southeastern Germany. One focus was detected when two human cases had occured in 2009 and 2010. In 2009 nine positive tick pools were detected by PCR in a total of 1,466 ticks. All except one positive tick pools were adult ticks. Until end of June 2010 in a total of 795 ticks in 140 tick pools four positive tick pools were detected, three of the four positive pools were from nymphs. In a second focus in 2009 two positive pools (all adult females) were found. In 2010, in 330 ticks eight positive pools were detected. Five of the eight positive pools were from nymphs and three positive pools with adult females were detected.
Our data for the first time show the dynamics of TBEV in ticks and their developmental stages in the longitudinal annual occurance. By comparison of the number of ticks and their infection rates an assessment of the relative risk of infection for the respective areas presuming constant human activities may become possible. Respective data on the activity of ticks and of TBEV in other endemic foci may be used for a nationwide surveillance system on TBE in Germany.

**Number E2**

## Seroprevalence of antibodies against Rickettsiae of the spotted fever group and of the typhus group in humans in Southwestern Tanzania

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Rickettsial infections in East Africa seem to be common. However, data on the prevalence rickettsial diseases in humans in countries of East Africa are rarely available. We therefore conducted a clinical study on the seroprevalence of IgG antibodies against rickettsial diseases in Tanzania. Human sera from nine different places with different altitudes (range form 500 to 1.700 meters) of the Mbeya district in Southwestern Tanania were tested against Rickettsia of the spotted fever group and of the typhus group by indirect immunofluorescence. A titer of 1:64 was considered to be specific and positive.
A minimum of 40 sera from each study site were tested. The sites differed by altitude above sea level and by structure (urban vs. rural). In all nine study sites the positive rates for rickettsiae of the spotted fever group were high and in some sites exceeding 70%. In contrast the prevelence of antibodies against was found to be much lower varying between 5 and 10%. The results imply that there is a high infection rate of rickettsiae of the spotted fever group in the ecological conditions tested in Mbeya region. Although, there are much lower prevalence rates of antibodies against the typhus group, infection with rickettsiae of this group could also be detected as evidence of low level transmission. Infection with rickettsiae of the spotted fever group seem to be ubiquitous while infections with rickettsiae of the typhus group seem to be more restricted to special circumstances.

**Number E3**

## Analysis of *Ixodes* and *Dermacentor* ticks for tick borne pathogens around Berlin

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We collected ticks (n=1038) in seven representative districts of Berlin. Two districts which represented the parkland within the city of Berlin were found completely free of ticks. In all other districts we regularly found ticks. *Ixodes* ticks of all stages were found in five out of seven districts. Adult ticks of *D. reticulatus* were found in three of seven districts, mainly in the southern districts. In one district more than 50% of the caught ticks were *D. reticulatus.*
Thirty ticks of every district were investigated for different pathogens by PCR (*Anaplasma, Babesia, Borrelia burgdorferi* and *Rickettsia sp)*. We analysed tick density and pathogen prevalence with a geographic information system (GIS) to gain an overview of the risk situation for every pathogen in the different areas.
In total 47% of all ticks were infected by *Rickettsia sp.* and 9% of all ticks were positive for *B. burgdorferi sensu lato.* Remarkably, *D. reticulatus* ticks were infected by *Rickettsia sp*. (*R. raoultii*) in most cases (66%) but not by *Babesia sp.* or *Anaplasma sp.* As much as 1% of *Rickettsia*-positive *Dermacentor* ticks were co-infected by *B. burgdorferi sensu lato*.
*I. ricinus* was found to be infected by *Rickettsia sp*. (30%), *Babesia sp*. (5%), and *Anaplasma sp*. (3%). A 16% of these *Rickettsia*-positive ticks were co-infected by *B. burgdorferi sensu lato.* No co-infections with *Anaplasma sp.* or *Babesia sp*. were observed.

Keywords: Dermacentor reticulatus, Ixodes ricinus, Rickettsia sp., Babesia sp., Borrelia burgdorferi sensu lato, Anaplasma sp.

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**Number E4**

## Clonal spread of Salmonella enterica serovar Derby from pigs to humans in Germany

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*Salmonella enterica* serovar Derby is one of the most prevalent serovars in pigs in Europe and ranks among the 10 most frequently isolated serovars in humans. Therefore, a set of 82 *S.* Derby strains isolated in 2006/2007 from pig, pork and human in Germany was selected and investigated for the transmission of clonal lines of the serovar along the food chain. Various phenotypic and genotypic methods were applied and the virulence and resistance gene repertoire was determined.
Phenotypically 72% of the strains were susceptible to all 17 antimicrobials tested while the others were monoresistant to tetracycline or multiresistant with different resistance profiles. Three major clonal lines were identified by sequencing data of the virulence genes *sopA*, *sopB* and *sopD*, VNTR-locus STTR5 and MLST. PFGE gave similar results. Thirty different PFGE profiles were detected resulting in four clusters of which two represented one lineage.
Overall this study showed that in Germany one major *S*. Derby clone is currently spreading in pigs and humans. Contaminated pork was identified as one vehicle and consequently presents a risk for human health. The virulence gene repertoire was found similar to *S*. Hadar, *S*. Virchow and *S*. Infantis. To prevent this serovar from entering the food chain, control measurements should be applied at pig farm level.

Keywords: *Salmonella*, serovar Derby, antimicrobial resistance, molecular typing, sequence typing

**Number E5**

## Current Q fever situation in Germany and in the Netherlands - epidemiological and zoonotic aspects -

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Q fever is a zoonotic infection caused by the bacterium *Coxiella burnetii*. Outbreaks in urban or residential areas have been recently reported in the Netherlands and Germany often involving large numbers of human cases. In most cases they were linked to small ruminant flocks. In the Netherlands the epidemic affected an area that has a high density of dairy goat farms. In 2009, human infections rise dramatically to more than 2,300 individuals. Therefore a nationwide hygiene protocol and a vaccination campaign with the Q fever vaccine "coxevac" was started for professional dairy goat and dairy sheep farms. In December 2009 almost 40,000 pregnant animals were killed. In Germany most of the human cases are caused due to infected sheep. 312 people fell ill in the year 2005 during a Q fever outbreak in Jena, a mid-size town in the state Thuringia, Germany. This was one of the largest Q fever outbreaks in Germany and linked to a herd of 500 sheep which were located near a suburb of Jena. In 2008/2009, a Q fever outbreak concerning 69 affected people was observed in the region of Freudenstadt (Schwarzwald). This outbreak was associated to wandering flocks of sheep. In contrast to the Netherlands the vaccine "coxevac" was not available in Germany.

Keywords: Q fever, goat, sheep, epidemiology, vaccination

**Number E6**

## Prevalence of zoonotic chlamydiae in dairy cattle and transmission to humans

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INTRODUCTION

Chlamydia infections in certain dairy farms are common. We want to find out whether they are related to chronic respiratory illnesses in farmers.

METHODS/MATERIAL

Nasal and vaginal swabs, milk and faecal samples and paired sera were obtained from 5 cows of each farm. Swabs, milk and faeces were examined by rtPCR and sera by ELISA. Farmers were clinically examined by lung function testing, serology (MIF), allergy skin test and their induced sputum was rtPCR tested.

PRELIMINARY RESULTS

Preliminary analysis of 46 dairy farms showed that in 26 (65%) farms at least 1 sample from at least 1 cow was positive in PCR. *Chlamydophila (C.) psittaci* and *C. ecorum* were mainly identified. 7 (15.2%) farms were positive only by ELISA. 11 out of 37 tested persons had respiratory symptoms and 33 persons were positive in serology. C*. pneumoniae* IgA occurred in 15 (40.5%) subjects and IgG in 26 (70.3%). Only 2 persons were positive for *C. psittaci* IgG. *Chlamydia trachomatis* was identified in 5 human sputum samples.

DISCUSSION

We found a wide dissemination of chlamydial infections in the cattle herds. Two of the *C. psittaci*-positive individuals had regular contact to birds. The presence of *Chlamydia trachomatis* in 5 sputum samples is remarkable and needs further investigation.

**Number E7**

## Alveolar echinococcosis as increasing risk for old world monkeys in a breeding enclosure

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The small fox tapeworm, *Echinococcus multilocularis*, causes the rare but severe zoonosis alveolar echinococcosis (AE) in humans. In the sylvatic life cycle, the parasite is transmitted from red foxes (*Vulpes vulpes*)- the final hosts- to voles- the intermediate hosts. Like humans, nonhuman primates may become aberrant intermediate hosts by ingestion of eggs and develop severe liver lesions caused by the infiltratively growing metacestode of the tapeworm.
Between 2000 and 2010, 33 monkeys (4 lion-tailed macaques, 23 cynomolgus monkeys, 6 rhesus monkeys) of the German Primate Center, Göttingen, were serologically tested positive for echinococcus antibodies. 19 of the affected animals died or were euthanized due to AE.
Gross pathology revealed hepatomegaly with granulomatous inflammation and fibrous transformation mainly in the right and median liver lobes. Cystic metacestode tissue with a weight up to 4000 g was found in the liver. In some animals, multilocular cysts were also present in the lungs, the pancreas and the heart. Histology revealed destruction of the liver parenchyma by the infiltrating cysts, which were surrounded by chronic inflammatory cell infiltration with foreign body type giant cells and contained multiple protoscolices.

The affected monkeys lived in an outdoor enclosure. Sources of infection may be feces of foxes rambling near the fences of the enclosure or contaminated branches from the adjacent forest, which are used for environmental enrichment in the enclosure.
From our data it can be concluded that nonhuman primates kept in outdoor enclosures of zoological gardens or other breeding colonies in the northern hemisphere are at high risk for AE.

Echinococcosis has to be considered as an emerging disease for susceptible animals in captivity.

Keywords: Fox tapeworm, alveolar echinococcosis, nonhuman primates, liver lesions

**Number E8**

## Bats and Herpesvirus-Infections – a model system for transmission dynamics of zoonotic viral agents?

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The role of bats (order *Chiroptera*) as reservoir of important zoonotic viruses has frequently been demonstrated. Yet, information on the ecology and dynamics of viral infections in these animals is still missing. The bats' intensive social interactions (e. g. communal roosting,) are an ideal foundation for establishing and distributing emerging infectious agents. Given species-specific differences in roaming behaviour and in the quality of their defence against pathogens, viral abundances should vary distinctively among bat species. In this regard, bats migrating long distances should differ from sedentary species.
In a pilot study, this relationship was exemplarily investigated with bat-specific herpesvirus infections as a model system. The prevalence of bat-specific herpesviruses was analysed in two European vespertilionid bat species from different locations in Germany. The large migrating Common Noctule (*Nyctalus noctula*) (n=65) revealed a significantly lower viral abundance than the smaller, sedentary Common Pipistrelle (*Pipistrellus pipistrellus*) (n=38). Yet, both share similar infection patterns concerning age and sex of host individuals. Interestingly, virus prevalence differed for both bat species depending on their originating region.
In conclusion, the bat-herpesvirus system might serve as a comprehensive model to study inter- and intraspecies transmission dynamics and modelling approaches might also allow estimations and extrapolation of transmission dynamics concerning emerging zoonotic viral agents in bat populations.

Keywords: Transmission dynamics, bat herpesvirus, modelling zoonotic viral infections, virus ecology

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**Number E9**

## Detection of co-infection of individual animals by different *Mycobacterium avium* subsp. *paratuberculosis* strains

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*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of paratuberculosis, a chronic gastroenteritis of cattle that is world-wide distributed and affecting a broad range of hosts. Its possible role as a zoonotic trigger for Crohn`s disease is under controversial discussion. Within the first funding period of ZooMAP a genotyping protocol for MAP with a high discriminatory power was established and evaluated and epidemiological links between cattle herds and between cattle and red deer were detected.
The aim of the current study was to find out, if individual animals can be co-infected by different MAP strains.
MAP isolated from two to six tissues per individual animal (14 cattle, 45 isolates) was genotyped using a combination of IS900-RFLP, VNTR-typing and MLSSR-sequence analysis. Animals originated from five German cattle herds.
As result, within different tissues of four individual animals of two herds different MAP-strains were detected. The originating tissue of the isolates differed from animal to animal, including ileum, jejunum, caecum, mesenterial lymph nodes, liver lymph nodes, muscle and faeces, and in one case between time points of sampling. Only one genotype was found for MAP isolated from tissues of animals of herd 3 to 5 (4 cattle, 14 isolates).
In conclusion, individual animals can be simultaneously infected by different MAP strains.

Keywords: MAP, paratuberculosis, Morbus Crohn, genotyping, co-infection

**Number E10**

## Risks and routes of chlamydial transmission evaluated in a bovine model of *Chlamydophila psittaci* infection

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**Aim:** Knowledge about a causative relationship between the presence of *Chlamydiaceae* in farm animals and transmission to potential hosts is lacking. This study verified shedding of *Chlamydophila* (*C.*) *psittaci* after experimental infection to evaluate risks and routes of chlamydial transmission.

**Methods:** Healthy calves (n = 21) where inoculated intrabronchially with $10^8$ inclusion forming units of *C. psittaci*, and were socialised with 3 naïve calves (sentinels) 2-3 days post inoculation (dpi). Spreading and shedding of chlamydiae was followed until 35 dpi by analysing venous blood, nasal and faecal swabs, exhaled breath, and room air using q rt-PCR and DNA microarray.

**Results**: After inoculation, faecal shedding of chlamydiae exceeded shedding *via* nasal secretions (total positivity rate in rectal swabs 39.6% for up to 18 dpi, in nasal swabs 7,4 % for up to 14 dpi). *C. psittaci* was detected in room air for at least 8 dpi, and in exhaled breath of an inoculated calf still 14 dpi. Sentinels acquired the infection. Systemic spreading of chlamydiae *via* blood continued for at least 30 dpi in inoculated calves and in sentinels, and was maximal within one week after challenge in both groups.

**Conclusions:**

Shedding of chlamydiae *via* the respiratory and the intestinal tracts resulted in ambient contamination and successful transmission of infection.

The presence of *C. psittaci* in peripheral blood indicates systemic spreading of a local infection after both induced and acquired infection.

**Number E11**

## Examinations of the tenacity of *Toxoplasma gondii* tissue cysts in vitro

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Introduction: Toxoplasmosis is one of the most common parasitic diseases. Humans are generally infected by the ingestion of tissue cysts within not sufficiently heat treated meat or meat products. But there are a plenty of not thermically treated meat products such as different raw sausages. In the study presented here, the conditions of raw sausage fermentation were simulated in vitro and the impact of single parameters was examined in respect of the infectivity of *Toxoplasma* tissue cysts.
Methods: The parameters pH-value and NaCl concentration were examined. For this purpose mice were infected orally with *T. gondii*. Six weeks p.i. muscles and brains of the mice were dissected and placed into RPMI medium with an adjusted pH of 5.0, 6.0 or 7.0. Furthermore, RPMI medium with adjusted NaCl or curing salt concentration of 2.0, 2.5 or 3.0 % was used. At the sampling days the material was fed to mice. After six weeks their brains were examined by microscopy and real-time PCR for the presence of *T. gondii*.
Results: Infectious parasites could be detected in the muscles for up to 26 days (pH 5.0) in brain for 28 days (pH 6.0). Tissue cysts remained infective at a NaCl concentration of 2.0 % for up to 8 days. Higher salt concentrations or the use of curing salt led to an earlier loss of infectivity.
Discussion: We could show that tissue cysts have a high pH-value tolerance. But on the other hand they are more sensitive against the salt concentration. Curing salt showed an even stronger effect concerning the damage of the tissue cysts than NaCl.
(This project is part of TOXONET, which is being supported by a grant from the BMBF)

Keywords: *Toxoplasma gondii*, raw sausage, tenacity, pH-value, salt concentration

**Number E12**

## Risk factors of sporadic Yersinia enterocolitica infections in Germany, 2009-2010: a case-control study

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Yersiniosis incidence in Germany is higher than in other European countries, highest in children aged <5 years and varies across federal states. We conducted a multi-state case-control study to investigate risk factors of sporadic yersiniosis. A case was defined as illness in a person with notified Y. enterocolitica infection between 15 April 2009 and 30 June 2010. Age-frequency-matched controls were randomly drawn from population registries. Participants completed self-administered questionnaires. Age- and sex-adjusted multivariable logistic regression models were constructed to identify possible risk factors. In preliminary analysis (501 cases and 1419 controls) the consumption of raw minced pork was the major risk factor of yersiniosis (adjusted odds ratio 4.0; 95% confidence interval 2.9-5.7). This association was even higher in children aged <5 years (6.1; 3.2-11.6). The proportion of cases reporting such consumption before disease onset was 32% overall, but also in children aged <5 years. Raw minced pork was most frequently consumed in federal states with high reporting incidences of yersiniosis. Additional risk factors were handling of raw minced pork in the household and, in children, playing in a sandbox. Reducing the high prevalence of Y. enterocolitica in pigs and pork products is critical for prevention of yersiniosis. Risks associated with consumption of raw minced pork need to be communicated more clearly to consumers, in particular to parents of young children.

Keywords: yersiniosis, risk factors, case-controls studies

**Number E13**

## Risk factors for *Toxoplasma gondii* infections in pigs in Lower Saxony, Germany

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Cats are definitive hosts of *Toxoplasma (T.) gondii*, i.e. they are able to shed environmentally resistant stages of the parasite, so called oocysts, in their feces. Oocysts are one of the sources of infection for intermediate hosts. Ingestion of raw or under-cooked meat, which contains infectious *T. gondii* tissue cysts, represents the predominant route for human infection. The aim of the study was to collect actual data on the seroprevalence and on potential risk factors for *T. gondii* infections in pigs in Lower Saxony. Within the frame of monitoring programs for Aujeszky´s Disease and Classical Swine Fever, 13,200 plasma samples from pigs were collected and examined in a commercial *T. gondii* ELISA. To determine risk factors, farmers who agreed to participate were interviewed in a telephone call. A mean sero-prevalence of 4% was determined for the pigs of 214 units, for which questionnaire data were available. At least one animal tested positive for antibodies to *T. gondii* in 22% of these units. The lowest prevalence was observed in units with fattening pigs (6%) and the highest in those with sows (34%). Potential risk factors for the infection of pigs with *T. gondii* were determined by logistic regression analysis. The results show that *T. gondii* infections are common in pigs, especially in older animals. Our results are of importance for the development of control strategies to prevent human *T. gondii* infection.
The study was funded by BMBF (01 KI 0765, Toxonet01).

Keywords: Toxoplasma gondii, pigs, risk factors

**Number E14**

## Detection of Rickettsia helvetica in parasites collected from free-ranging mammals – unraveling hosts putatively involved in the rickettsial life cycle

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The epidemiology and ecology of most rickettsia, particularly their transmission cycle and the role of vertebrate hosts in maintaining rickettsia, is not completely known. Aim of our study was to clarify the role of vertebrates in the natural transmission cycle of Rickettsia spp. Parasites were collected from 33 European hedgehogs, 33 roe deer, 4 red deer, 1 red fox, 1 European brown hare, and 2 wild boars. Ixodes (I.) ricinus (n=1109), 555 I. hexagonus, 24 Haematopinus suis, 30 Lipoptena cervi, and 38 deer botfly larva were investigated for the presence of rickettsia by real-time PCR targeting gltA. Rickettsial species were identified by sequence analysis of the ompB gene. In addition, DNA from tissues of European hedgehogs was investigated. Rickettsial DNA was detected in I. ricinus and I. hexagonus, 1 deer botfly larva, and 2/11 tissue samples from European hedgehogs. OmpB sequence analysis revealed 99-100% similarity to R. helvetica. In ixodid ticks from 22/33 (67%) hedgehogs R. helvetica DNA was found. The minimum infection rate (MIR; 12.5%) did not differ significantly between I. ricinus and I. hexagonus. PCR-positive I. ricinus (MIR=13%) were collected from 19/33 (58%) roe deer. Ixodes ricinus (n=19) collected from a European brown hare revealed a MIR of 26%. Ticks collected from red foxes and red deer were negative for Rickettsia DNA by PCR. In conclusion, R. helvetica seems to be maintained in parasites from small mammals and wild ungulates. The detection of Rickettsia DNA in tissues from European hedgehogs suggests that these mammals play an important role in the natural cycle of R. helvetica.

Keywords: Rickettsia helvetica, epidemiology, wildlife

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**Number E15**

# Risk factor analyses of the serological prevalence of *Yersinia enterocolitica* in fattening pig herds

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*Yersinia* (*Y.*) *enterocolitica* presents a common cause of human gastroenteritis. Pigs have been identified as the primary reservoir of pathogenic *Y. enterocolitica* and food products of porcine origin are an important source of human infection. To estimate serological prevalence within 80 fattening pig herds, blood samples from 30 pigs of each herd were taken and tested for antibodies against pathogenic *Yersinia* strains (Pigtype Yopscreen ELISA, Labordiagnostik Leipzig, Germany).
The serological within-herd prevalence varied from 0 % to 100 %, indicating that specific farm factors affect the prevalence of *Y. enterocolitica* in the pig herds. Because of a bimodal distribution, herds with a prevalence of 20 % and more were considered as positive. Association between farm factors and serological prevalence were analysed by logistic regression.
High weight gain, the origin of drinking water, the occurrence of herd diseases and the structure of the floor had an influence on the serological prevalence of *Y. enterocolitica*.

Keywords: Yersinia enterocolitica, risk assessment, fattening pigs, with-in herd prevalence

**Number E16**

## *S. pseudintermedius*-carriage in dogs: A potential risk for dog's best friends?

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Among commensal bacteria of the skin and mucosa in dogs, *Staphylococcus pseudintermedius* is one of the most important opportunistic pathogens worldwide. Although the incidence of infections in animals is constantly rising due to multidrug- and methicillin- resistant *S. pseudintermedius* (MRSP), serious infections in humans have been reported only recently. Since the socio-economic relationship between the majority of dogs and their owners has changed dramatically during the last decades from working dogs (watch dogs, sheepdogs etc.) living in stables or kennels to pets living in the household almost as family members, there might be an elevated risk for the transmission of canine microorganisms to humans.

To get first insights into the proportion of both dog owners and their healthy animals colonised with *S. pseudintermedius*, we conducted a screening study during a dog show event in Berlin in February 2009 with a total of 110 voluntary attendees.

Among the sampled dogs, 15 (13.6%) were colonized with *S. pseudintermedius*; none of the isolates proofed to be MRSP. We found 5.5% (n= 6) of the dog owners being nasally positive for *S. pseudintermedius*, including one case of MRSP belonging to multilocus sequence type (ST) 41. In addition, pulsed-field gel electrophoresis (PFGE) revealed simultaneous colonization of one dog and his owner with PFGE-indistinguishable *S. pseudintermedius* isolates (ST33). Furthermore, we found two isolates sharing identical PFGE patterns in samples of one other person and a dog with no known relationship.

According to our results, close side-by-side living with a dog may lead to a risk for colonisation with *S. pseudintermedius* in humans. Further epidemiological investigations are necessary,

especially in view of the increasing numbers of MRSP- infections in humans.

Keywords: *S. pseudintermedius*, MRSP, dog, human, colonization

**Number E17**

## Case-Control Study on Risk Factors for Sporadic Salmonellosis in Lower-Saxony, Germany

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Background:
Salmonellosis is the second most common reported bacterial human intestinal disease in Germany. In Outbreaks, the joint exposure of the cases is often obvious, or the source of infection can be identified by microbiological investigation of left-over food items or by epidemiological outbreak investigations. Besides products from pork or eggs many other sources have been identified in this way, including tomatoes, milk products, peanut butter, food for pets or contact with reptiles. However, about 80% of the reported *Salmonella*-infections from humans are sporadic cases. For these sporadic infections the source of infection usually remains unknown. With the aim to identify risk factors for sporadic *Salmonella*-infections, a case-control study was conducted in Lower Saxony (Germany).
Methods:
We stratified the cases and controls by age groups. Here we only report on the subset of adult participants, i.e. cases and controls older than 14 years.
The study cases were selected from *Salmonella*-cases reported according to the German infectious disease notification system from June 2008 to May 2010 in Lower Saxony. Notified *Salmonella*-cases, which were laboratory confirmed and fulfil the German surveillance case definition where included, if the patients could get contacted by phone, gave their informed consent and were able to complete a telephone interview. Cases that were obviously part of an outbreak were excluded.
Randomly selected controls from the general population (population-controls) were enrolled by random digit dialling, i.e. randomly dialling phone numbers from Lower Saxony according to the Gabler-Häder-Design. From one household the person with

most recent birthday was chosen and included if the person agreed to do a telephone interview.
A second group of controls was enrolled from reported cases with Rotavirus-infection (Rotavirus-controls). Persons with reported Rotavirus-infection, who were not part of a reported outbreak, were asked for consent for participation in the study. Persons who were not able or not willing to complete a telephone interview were excluded.
For data collection a standardised questionnaire was used. The questionnaire provided information about consumed foods, hygienic aspects of food handling in the kitchen, contacts with animals and other activities during the last three days before onset of disease. Population-controls were asked about the three days before the interview. The questionnaire also provided information on other conditions possibly associated with *Salmonella*-infection, e.g. diabetes, immune deficiency, use of drugs.
The associations between outcome (disease status) and exposures were assessed by multiple logistic regression analysis with a stepwise variable selection procedure. The results are presented as odds ratios, adjusted for season and other exposures (aOR), accompanied by 95%-confidence-intervals (CI) and two-sided p-values for the Wald-test, where $p<0,05$ was assumed to indicate a significant association. The multiple logistic regression analysis was done separately for each group of controls.
Results:
The study population finally contained 1017 cases (471 males, 546 females), 173 population-controls (92 males, 81 females) and 170 Rotavirus-controls (84 males, 86 females) aged 15 years and older with a successfully completed interview. The mean age was 44,2 for cases and 47,9 and 46,7 years for the two groups of controls, respectively.
By multiple logistic regression analysis, 13 variables were identified which were most likely to have a major association with disease status.
With respect to the population-controls, significant associations were detected between Salmonellosis and consumption of raw pork (aOR 2,39; CI 1,28-4,47), travelling abroad (aOR 2,35; CI 1,11-5,00), barbecuing (aOR 4,40; CI 1,57-12,32), consumption of game (aOR 1,85; CI 1,06-3,22) within 72 hours before onset of disease and taking antacids during the last 4 weeks before onset

of disease (aOR 2,26; CI 1,11-4,63). A significant negative association was observed with animal contact (aOR 0,58; CI 0,41-0,82) and consumption of uncooked tomatoes, pepper and herbs (aOR 0,53; CI 0,35-0,80). No significant association could be found with any of the variables referring to hygienic behaviour.
Comparing cases with Rotavirus-controls leads to similar results regarding consumption of raw pork (aOR 4,90; CI 2,11-11,38) and travelling abroad (aOR 2,17, CI 1,08-4,37). There was no significant association for barbecuing, consumption of game, consumption of uncooked tomatoes, pepper and herbs and animal contact. Consumption of eggs (aOR 2,06, CI 1,23-3,47) and job related animal contact (aOR 2,98, CI 1,18-7,53) was significantly associated with disease status. No association with antacids was found for this group of control persons.
Conclusions:
In the case-control study several exposures could be identified which positively or negatively were associated with case status, mainly food items.
The distribution of age and gender was similar in the case- and control populations. Thus, there was no evidence for selection bias with respect for these demographic factors. Also recall bias is often a source of error in case-control-studies, if diseased cases are more likely to remember exposures than controls. We dealt with this problem asking the population-controls for exposures during the most recent 72 hours, and using a second control group who experienced similar disease as the cases and therefore should remember their consumed food with similar precision. Confounding and multicollinearity among exposure variables were considered by fitting a multiple logistic regression model. Nonetheless, these factors had to be watched carefully when interpreting the results.
Consumption of raw pork products and travelling abroad were major risk factors for sporadic Salmonellosis. The impact of consumption of products containing raw eggs or other poultry products is smaller than expected, possibly because of control measures in layer hens during the last years.
We could not identify any association between hygienic deficiency in the kitchen and *Salmonella*-infection. This result may be due to social desirability bias, i.e. cases who feel guilty of hygienic deficiencies are more likely to tell the untruth than healthy controls. Contact with animals was negatively associated with

*Salmonella*-infection. The reason for this result has to be evaluated in further research.
Different results with respect to the two groups of controls (i.e. taking antacids) could be explained by differences in pre-existing medical conditions or differences in health care seeking behaviour between Rotavirus-controls and population-controls.

Keywords: *Salmonella*-infection; food; antacids; infectious disease surveillance

# Poster Session – Immunity and Pathogen-Host-Interaction

**Number I1**

## Establishment of a murine infection model for *Mycobacterium avium* ssp. *paratuberculosis*

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*Mycobacterium avium ssp. paratuberculosis* (MAP) is a very slow growing, acidfast bacterium which is responsible for Johne´s disease, a chronic enteritis in ruminants. There is also evidence that MAP might be implicated in Crohn´s disease in humans. Biopsies of many patients suffering from this disease contained MAP.

MAP is most likely taken up by the oral route and can colonize gut-associated macrophages. It is known to be able to survive and multiply in such cells. However, until now the way of infection, the survival of MAP in the host and the raise of inflammation are not clearly understood. One reason for this is the lack of a suitable mouse model for detailed investigations.

The aim of this project is to establish a murine infection model with immune deficient mice and to monitor the entry and the infection process of MAP in such animals.

New born or adult wildtype, $RAG^{-/-}$ and $RAG^{-/-}c\gamma^{-/-}$ mice were either orally or intraperitonealy (i.p.) infected. $RAG^{-/-}$ mice lack mature T- and B-lymphocytes. $RAG^{-/-}c\gamma^{-/-}$ additionally lack NK-cells. Oral infection was only marginal successful in newborn wildtype or knockout mice. In contrast in 80% of i.p. infected wildtype or immune deficient mice MAP could be detected by PCR and histology. But only i.p. infected wildtype mice had spleens twice as big as uninfected controls.

To check the influence of T cells, i.p. infected immune deficient mice were reconstituted with sorted $CD4^{+}CD45RB^{low/med}$ T-cells from spleens of uninfected wildtype mice. The reconstitution of the immune system after infection with MAP should mimic physiological conditions. $CD4^{+}CD45R^{high}$ T-cells are known to elicit colitis when they are transferred into $RAG^{-/-}$ mice. Interestingly no granulomas in liver sections from MAP i.p. infected immune deficient mice were found. But the bacteria were detected by PCR. After i.p. infection and reconstitution, granulomas were visible in liver sections and PCR was positive for MAP. Additionally spleens from these mice were twice as big as spleens from not

reconstituted mice. These results are comparable to the observation in i.p. infected wildtype mice.
Further experiments should answer the question which additional cells are involved in the immune reaction against MAP. Moreover fluorescent MAP will be monitored shortly after i.p. infection.

Keywords: Johne´s disease; Crohn´s disease; *Mycobacterium paratuberculosis*; Mouse model

**Number I2**

## Development of a bat interferon specific bioassay

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Zoonotic viruses like Ebola- and Marburgvirus, Nipah virus and SARS-Coronavirus are most likely hosted by bats and responsible for severe human diseases. Differences in the innate immunity between humans and bats can be assumed. The interferon (IFN) system plays a major role in antiviral defense. It is activated upon recognition of viral dsRNA or dsDNA by different cytosolic receptors. The expressed IFN is secreted in by auto- and paracrine mechanism causing an antiviral state. IFN detection assays are not commercially available for bat cells.
Here we designed assays for investigating induction, secretion and signalling of bat IFN (bIFN). To be able to measure bIFN we applied a classical vesicular stomatitis virus bioassay on different bat cells (*Rousettus aegyptiacus* and *Eidolon helvum* kidney cells). Standard curves were established with a pan-species recombinant IFN (rIFN).
For assay evaluation bIFN containing supernatants were produced by stimulating bat cells with poly (I:C), Rift Valley Fever virus-clone 13 and O´nyong-nyong virus. Subsequently, for virus inactivation, the supernatants were treated with beta propiolactone. In all samples the amount of bIFN could be calculated relative to rIFN in a range of 1 to 100 U per ml.
The developed assay allows us to examine the differences between human and bat innate reactions to viruses on the IFN secretion level.

Keywords: bioassay, interferon, bat

**Number I3**

## Expression of CD25 on T-memory cells: Biomarker to detect calves infected with *Mycobacterium avium* subsp. *Paratuberculosis* (MAP)

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Identifying infant cattle infected with MAP remains a difficult task. Aim of this study was to assess whether the quantification of activation markers on the surface of T-cell subsets allows the identification of MAP-infected calves. Six calves, experimentally infected with MAP, and six control animals were sampled (whole blood) on a regular basis over a 12 months period. Peripheral mononuclear cells (PBMC) were incubated for 5 days in vitro with whole cell sonicates (WCS) derived from MAP, M. avium subsp. avium (MAA) and M. phlei (MP), respectively. Expression (mean fluorescence intensity) of CD25 and CD26 on the surface of lymphocyte subsets was quantified via flow cytometry. In the MAP-infected group, incubation with WCS-MAP led to a significant increase of CD25 expression on $CD4^+$ and $CD8^+$ T-memory cells ($CD45RO^+$) as well as on γδ-T-Zellen ($TcR1\text{-}N24^+/CD2^-$) over time and in comparison to the control group ($p < 0.001$). CD26 expression on $CD4^+$ and $CD8^+$ T-memory cells and CD25 expression on NK-cells ($CD335^+/CD2^+$) was not significantly different between the groups. Incubation with WCS-MAA and WCS-MP resulted in minor or no increased expression of the activation markers. When analyzing the CD25 expression on $CD4^+/CD45RO^+$ cells and based on a calculated cutoff, 92 % and 97 % of all samples of the MAP-infected calves were classified as positive 12 and 16 weeks post infection, respectively. Thus, this approach will be instrumental for the early diagnosis of bovine paratuberculosis.
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infected with MAP, and six control animals were sampled (whole blood) on a regular basis over a 12 months period. Peripheral mononuclear cells (PBMC) were incubated for 5 days *in vitro* with whole cell sonicates (WCS) derived from MAP, *M. avium* subsp. *avium* (MAA) and *M. phlei* (MP), respectively. Expression (mean fluorescence intensity) of CD25 and CD26 on the surface of lymphocyte subsets was quantified via flow cytometry. In the MAP-infected group, incubation with WCS-MAP led to a significant increase of CD25 expression on CD4+ and CD8+ T-memory cells (CD45RO+) as well as on γδ-T-Zellen (TcR1-N24+/CD2-) over time and in comparison to the control group ($p < 0.001$). CD26 expression on CD4+ and CD8+ T-memory cells and CD25 expression on NK-cells (CD335+/CD2+) was not significantly different between the groups. Incubation with WCS-MAA and WCS-MP resulted in minor or no increased expression of the activation markers. When analyzing the CD25 expression on CD4+/CD45RO+ cells and based on a calculated cutoff, 92 % and 97 % of all samples of the MAP-infected calves were classified as positive 12 and 16 weeks post infection, respectively. Thus, this approach will be instrumental for the early diagnosis of bovine paratuberculosis.

Keywords: cellular immune response, bovine paratuberculosis, flow cytometry, $CD4^+$-cells, CD25

**Number I4**

## Quantification of intracellular IFN-γ to detect antigen-specific CD4$^+$ T-cells in early stages of bovine paratuberculosis

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Quantification of extracellular IFN-γ in antigen-stimulated whole blood samples to detect pathogen-specific cellular immune responses often results in false positive reactions when applied to juvenile animals. We aimed to assess whether intracellular detection of IFN-γ in selected T-cell subsets may lead to a reliable identification of juvenile cattle infected with *Mycobacterium avium* ssp. *paratuberculosis* (MAP). Six MAP-negative calves and 6 calves orally inoculated with MAP at 10 days of age were sampled every 4 weeks for 12 months. Isolated blood mononuclear cells were stimulated with either purified protein derivatives (PPD) or whole cell sonicates (WCS) derived from MAP, *M. avium* ssp. *avium* (MAA) or *M. phlei* (MP) for 6 days followed by labeling of intracellular IFN-γ in both CD4$^+$ and CD8$^+$ T-cells and subsequent flow cytometric analysis. In comparison to WCS, responses to PPD were not as consistent. No antigen specific IFN-γ production was detectable in CD8$^+$ T-cells. In contrast, CD4$^+$ T-cells of 4/6 MAP-infected calves responded to WCS-MAP 16 weeks post inoculation. During the whole observation period, at least three samples of any of the infected calves tested positive. Reaction titers tended to decline by the end of the observation period. False positive reactions occurred in the control group with a frequency of 5 %. In conclusion, MAP-antigen specific IFN-γ production in CD4$^+$ T-cells may serve as a useful diagnostic tool to identify MAP-infected juvenile bovines.

Keywords: cellular immune response, bovine paratuberculosis, flow cytometry, CD4$^+$ cells, IFN-γ

**Number I5**

## Detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)-specific antibodies in cattle via flow cytometry

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In order to improve MAP-serology in cattle, the aim of the study was to develop a new flow cytometric (FC) based method for the detection of MAP-specific antibodies. Serum samples were collected from adult cattle (18 MAP-shedders, 22 MAP-negative) aged over 2 years as well as from calves (6 MAP-infected and 6 controls), which were sampled on a regular basis for 12 months. After preadsorption with *M. phlei*, serum samples were incubated with viable MAP and *M. avium* subsp. *avium* (MAA) bacteria, respectively, followed by the detection of IgG, $IgG_1$, $IgG_2$, and IgM attached to the cell surface. After FC analysis, MAP-specific antibody titres were calculated. Test results were compared to commercially available ELISA systems. In adults and defining specificity as 100 %, the FC-method ($IgG_1$-detection) demonstrated a sensitivity of 83 %. The sensitivity of the Cattletype®- and the Pourquier®-ELISA were 61 % and 72 %, respectively. In calves and based on a calculated cutoff, elevated antibody titres (IgG- and $IgG_1$-detection) were detectable in 2 out of 5 MAP-infected animals from 44 and 46 weeks post infection (w.p.i.) onwards, respectively. None of the MAP-infected calves showed elevated $IgG_2$- or IgM-antibody titres during the observation period. The Pourquier®-ELISA detected one MAP-infected calf as test-questionable from the 50 w.p.i. onwards. In conclusion, the FC-method proved more sensitive than the commercial assays but MAP-serology in calves remains a challenge.

Keywords: humoral immune response, bovine paratuberculosis, flow cytometry

**Number I6**

## Characterization of the ExPEC Adhesin I (EA/I) of avian pathogenic *E. coli* (APEC)

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Avian pathogenic *E. coli* (APEC) are a principal cause of morbidity and mortality in poultry worldwide. A previously performed signature tagged mutagenesis applied to APEC strain IMT5155 (O2:K1:H5; multilocus sequence type complex 95), in a chicken lung colonization model resulted in the identification of a novel adhesin gene (*eaID*), which is part of a 5 kb operon coding for a putative adhesive fimbria. Indeed, when the *eaI* operon was expressed in the afimbriate *E. coli* AAEC189, short fimbrial-like appendages could be seen under the electron microscope. Interestingly, we observed that *eaID* is not only highly prevalent among APEC but also among human extraintestinal pathogenic *E. coli* (ExPEC) pathotypes, including those associated with urinary tract infections and newborn meningitis, while it was absent in all of the intestinal pathogenic *E. coli* strains tested. This prompted us to designate the adhesin ExPEC Adhesin I (EA/I).
Certain ExPEC subgroups are known to have a low host-specificity and therefore a high zoonotic potential. Thus, it is of interest to investigate the role of EA/I fimbriae in APEC infection since it could be likewise significant for other ExPEC infections.
Here, we present first functional studies showing that EA/I-fimbriae are involved in hemagglutination of chicken, rabbit and horse erythrocytes; in motility and are associated with higher serum sensitivity. Another central aspect of our work is the identification of the receptor interacting with EA/I, in the respective host.

Keywords: ExPEC, APEC, Fimbriae, Adhesin

**Number I7**

## MHC I cross-presentation in chlamydia- infected dendritic cells

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To investigate MHC I-presentation of chlamydial antigens in dendritic cells (DCs), we performed infection studies using an immortalized DC cell line. We demonstrate that upon chlamydial infection surface presentation of MHC I is up-regulated in DCs. This is accompanied by an increased expression of intracellular MHC I and the chaperone tapasin that controls MHC I assembly and maturation. In contrast, no comparable stimulation was observed for the peptide transporter TAP, which is functionally essential for the classical antigen presentation. By immunofluorescence studies we observed co-localization of chlamydial vacuoles with endosomes containing cathepsin proteases, which are believed to play an essential role in the antigen processing of the vacuolar MHC I cross-presentation. Furthermore, electron microscopic analysis indicates that chlamydial inclusions are structurally dissolved in DCs and that chlamydial antigens are processed in lysosomal compartments. Most importantly, our studies revealed that during DC-infection post-ER MHC I molecules are translocated from the Golgi/TGN to cathepsin containing compartments indicating for the first time that DCs are able to cross-present chlamydial antigens via a TAP-independent vacuolar pathway.
On the basis of our findings we propose that vacuolar MHC I cross-presentation plays key role in DC-mediated processing of chlamydial antigens.

Keywords: antigen presentation, MHC I, dendritic cells, Chlamydia, vacuolar cross- presentation

**Number I8**

## Coronavirus but not Paramyxo-, Rhabddo-, and Orthomyxovirus entry in bat cells is restricted by the lack of appropiate receptors

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Coronavirus (CoV) infection starts with the binding to a receptor molecule on the cell surface and is mediated by the spike glycoprotein. In contrast to other viruses like influenza or Sendai virus, that infect a broad range of mammalian cell lines, CoV show a strong species specificity that relies on the presence of a proper receptor molecule on the host cell like the angiotensine converting enzyme 2 (ACE2) for the severe acute respiratory syndrome associated CoV (SARS) or the porcine aminopeptidase N (pAPN) for transmissible gastroenteritis virus (TGEV).
To investigate the crucial role of the CoV spike protein for virus entry we tested the ability of CoV to infect different bat cell lines, as there is strong evidence that bats can serve as a natural reservoir for CoV. We chose TGEV and a vesicular stomatitis virus pseudotyped with the spike glycoprotein of SARS and infected bat cell lines that were transfected with plasmids encoding ACE2 or pAPN prior to infection. As controls we used paramyxoviruses (Sendai virus, SeV; bovine respiratory syncytial virus, BRSV), a rhabdovirus (vesicular stomatitis virus, VSV), and an orthomyxovirus (influenza A, subtype H9N2) which are known to infect different mammalian cell lines.

Keywords: Coronavirus, entry, spike protein, receptor, bats

**Number I9**

## Organ distribution of *Chlamydophila psittaci* after intrapulmonary inoculation in calves

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**Aim:** In order to understand how systemic spread of *Chlamydia* spp. in the host organism contributes to shedding and transmission of the bacteria, the organ distribution of *Chlamydophila (C.) psittaci* was investigated in a calf model.
**Methods:** Conventionally raised calves (n=21) were inoculated intrabronchially with $10^8$ inclusion-forming units of *C. psittaci* of bovine origin. Three calves each were necropsied at 2, 3, 4, 7, 10, 14 and 35 days post inoculation (dpi). Representative tissue samples (47/calf) were collected for histology as well as immunhistochemistry (ihc) and quantitative real time (q rt-) PCR to detect chlamydiae. A DNA microarray was used for species differentiation.
**Results:** Acute fibrinopurulent bronchopneumonia with necrosis and numerous chlamydiae was seen in all calves at 2, 3, 4 and 7 dpi by ihc. Lesions became smaller and were demarcated by lymphocytes and macrophages at 10, 14 and 35 dpi. Detection of chlamydiae was restricted to areas of necrosis. Using q rt-PCR, chlamydiae were detected in pulmonary tissues of all calves, as well as in bronchial and mediastinal lymph nodes from 2 to 10 dpi. In addition, chlamydiae were detected in the upper respiratory tract, synovialis, aorta and ileum of individual calves.
**In conclusion**, the infection remained mainly limited to the lung, where it persisted until 35 dpi. Although dissemination beyond the respiratory system was rarely observed, it might contribute to systemic spreading of the infection.

**Number I10**

## The contribution of *Salmonella enterica* molecular patterns to NF-κB activation in different host species

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Nuclear factor-kappa B (NF-κB), is a key regulator of host cell immune responses to bacterial infections. NF-κB activation is induced by a variety of pathogen-associated molecular patterns (PAMPs) recognized by Toll-like receptors (TLRs) and cytosolic NOD-like receptors (NLRs), including flagellin (TLR5), LPS (TLR4/MD2) and bacterial cell wall muropeptides (NOD1 and NOD2). In mice, aflagellar *Salmonella* induce less proinflammatory and anti-apoptotic signalling leading to stronger inflammation and mucosal destruction.
To characterize host-specific differences in the activation of NF-κB in response to infection with *Salmonella* serovars, we constructed chromosomally integrated NF-κB reporter fusions in epithelial and macrophage-like cell lines of porcine, human and chicken origins. We compared the NF-κB activation in response to wild-type and strains of *S.* Typhimurium harboring Δ*fliC*Δ*fljB* (flagellin), Δ*ampG*Δ*mppA* (muropeptide uptake and recycling) and Δ*mepA* (peptidoglycan endopeptidase) mutations.
The various host species showed strong differences in NF-κB activation in response to flagellin or muropeptides. The human cell line showed elevated NF-κB activation after an infection with Δ*ampG*Δ*mppA* and reduced NF-κB activation for a Δ*fliC*Δ*fljB* mutant strains whereas these PAMPs did not appear to contribute to NF-κB activation in macrophage-like cells and epithelial cells of porcine origin. The results suggest host-specific differences in innate immune recognition of *Salmonella*.

Keywords: Salmonella, NF-κB, host-adaptation, immune response, flagellin

**Number I11**

## The role of the effector protein AvrA in host adaptation by *Salmonella enterica* serovars

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AvrA is a *Salmonella* virulence effector protein present in 80% of *Salmonella enterica* serovars and which is translocated into host cells by a type III secretion system. AvrA has been postulated to be a virulence factor by some authors where it could play an important role in modulation of the host inflammatory response. Although its exact function is not entirely clear, AvrA belongs to the family of cysteine proteases regulating diverse bacterial-host interactions and 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. The goal of this project is to clarify how this virulence factor contributes to the pathogenesis and host adaptation of these serovars, in particular the swine-adapted serovar, *S*. Choleraesuis.
We compared host cell invasion and intracellular growth of broad host-range serovars *Salmonella* Typhimurium and *S.* Enteritidis which harbor the *avrA* gene, with the host-adapted serovar, *S.* Choleraesuis, in human and porcine intestinal epithelial and macrophage cell lines. In standard invasion assays, *S*. Typhimurium showed both an apparent higher rate of host cell invasion, as well as a higher rate of intracellular proliferation than *Salmonella* Choleraesuis. However, in time-course infection studies, we found that the intracellular growth rate of *S*. Choleraesuis was identical to that of *S*. Typhimurium, the apparent lower proliferation of S. Choleraesuis was apparently due to earlier release of intracellular bacteria, *i.e*. earlier onset of host cell killing.
To determine whether the absence of the *avrA* gene in S. Choleraesuis was responsible for the apparent earlier induction of host cell death, we constructed mutants of the *avrA* gene in *S*. Typhimurium and other serovars for comparison with *S.*

Choleraesuis. No differences in the rate of cellular invasion between *S.* Typhimurium wildtype and Δ*avrA* mutants were observed. In addition, we have cloned the *avrA* gene from strains known to express the protein, and plan to introduce it into *Salmonella* Choleraesuis in order to determine whether this virulence factor is involved in the difference in the pathology caused by these serovars in humans and swine.

Keywords: Salmonella enterica, effector protein, AvrA, host adaptation

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**Number I12**

## Influence of SARS-related bat-Coronavirus ORF6 on virus replication in primate and bat cell culture

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Bats have been identified as a likely reservoir of Coronaviruses, including Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV). Recently our group was able to sequence the whole genome of a European SARS-related bat-CoV (BtCoV) out of Rhinolophus blasii faeces. SARS-CoV encodes for several proteins evading the innate immune response (non-structural proteins 1 and 3, open reading frames 3b and 6, and the nucleocapsid). The ORF6 protein recruits Karyopherin β1 and retains it on the ER/Golgi membrane thus inhibiting the transport of the interferon-stimulated gene factor 3 (ISGF3) complex into the nucleus.
In the present study we investigated the influence of the BtCoV ORF6 protein on the IFN system of human and bat cell culture.
Using the SARS-CoV reverse genetic system we created a SARS-CoV containing the BtCoV orf6 (BG-ORF6-rSCV). We then infected primate cells and bat cells carrying the human SARS-CoV receptor angiotensin converting enzyme 2 (ACE2) with wild type recombinant SARS-CoV (rSCV) and BG-ORF6-rSCV. Even under interferon (IFN) stimulation BG-ORF6-rSCV grew to higher titers than the wild type virus on either cell line.
This finding suggests that the IFN antagonist orf6 does not need host adaptation in order to function in other species. Future studies will investigate if BG-ORF6 has the same mechanism of action as its equivalent SARS-ORF6 in primate and bat cells.

Keywords: SARS-CoV, SARS-related bat-CoV, ORF6

**Number I13**

## Tools to characterize the type I interferon system in bat cells

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Bats have been identified as candidate reservoirs of many human-pathogenic viruses, e.g. SARS-Coronavirus. They seem to develop no clinical symptoms upon infection with highly pathogenic viruses. Differences in the immune system of bats versus other mammals have been suspected. One major antiviral defense is the type I interferon system, which comprises the sensing of cytoplasmatic nucleic acids, the production of interferon, and the expression of interferon-stimulated genes (ISG) causing an antiviral state. With the use of low stringency PCRs we could amplify critical genes for these steps in different bat species. These genes were deviant against homologous genes of other mammals, but were generally conserved within the order chiroptera. For a rough transcription profiling, these sets of genes were targeted by real-time RT-PCRs. For evaluation, different bat cells were transfected with both poly(I:C) and 5´-triphosphate RNA, or were infected with Rift Valley Fever virus-clone 13. The transcription of bMxA, bISG56 and bCCL5 strongly increased after stimulation. Increase of expression of these genes was confirmed by immunoblotting. As in human cells, SARS-CoV efficiently inhibited type I interferon response in human-ACE2-transfected bat cells. With these assays we now have tools to investigate virus-host interactions in greater detail.

Keywords: Interferon, Bats, SARS, qRT-PCR

**Number I14**

## Induction of specific IFN-γ secreting immune cells and antibodies in goats after experimental infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

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Development of novel diagnostic tests for paratuberculosis requires a reproducible animal infection model. To establish a model in goats we monitored the course of humoral and cellular immune responses relative to different modes of inoculation.
Two groups were inoculated orally ten times every other day beginning at day three of life with 10 mg and 20 mg of MAP, respectively. Another two groups were inoculated beginning at day 42. PBMC were prepared monthly and re-stimulated with purified protein derivative from MAP (PPDj), avian PPD or recombinant MAP proteins. Concentrations of secreted Interferon-γ (IFN-γ) were measured with an in-house ELISA. Antibodies were quantified in serum by commercial ELISA.
Beginning with week 12 p.i., re-stimulated PBMC from inoculated goats produced significantly more IFN-γ than cells from non-inoculated goats. Re-stimulation with PPDj led to higher IFN-γ responses than re-stimulation with avian PPD. None of the recombinant MAP proteins induced IFN-γ secretion. Animals inoculated with the lower dose beginning at day 42 showed a significantly smaller in-group variability than animals infected earlier or with the high dose. Specific antibodies were detectable from week 16 p.i. onwards in all groups.
Inoculation of goats with 10 mg of MAP from day 42 of life onwards proved to be the most suitable mode of inoculation to reproducibly induce an immune response. Further studies deploying this model will reveal underlying cellular and mediator responses.

Keywords: paratuberculosis, animal model, Interferon-gamma assay, antibody response, goats

**Number I15**

## Interaction of tick-borne encephalitis virus with human myeloid- and plasmacytoid dendritic cells

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Tick-borne encephalitis virus (TBEV) is the most prevalent arbovirus in Europe and in many parts of Asia. Clinical symptoms range from mild fever to severe neurological disorders. However, the pathogenesis of TBEV is still not completely understood, mechanisms of virus host response interaction and principles of neuroinvasion remain to be elucidated. Dentritic cells (DCs) are the most potent antigen-presenting cells and very important components for the immune system in terms of infections with pathogens including viruses. Corresponding to their function DC are divided into two main subtypes, myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). Whereas activated pDCs play an important role as main type I interferon (type I IFN)-producing cells and represent a link of innate and adaptive immune response, mDCs are the main antigen-presenting DC subtype playing a decisive role in the adaptive immune response. We analyzed the interaction of human mDCs and pDCs with the highly virulent TBEV strain Hypr. TBEV infected pDCs produced high amounts of interferon- (IFN-) α as well as inflammatory cytokines interleukin-6 (IL-6), IL-8 and tumour necrosis factor-α (TNF-α). At the same time pDCs exhibited only a transient upregulation of surface molecule markers (adhesion markers CD54, CD58, maturation marker CD83, co-stimulatory and activation markers CD40, CD80, CD86, apoptosis marker CD95 as well as antigen-presenting complexes MHCI and MHCII) but were capable to restrict TBEV replication. This restriction could be overcome by preincubation of pDCs with an antibody blocking the interferon α/β receptor indicating that the high levels of IFN produced by pDCs protect themselves from TBEV infection.

In contrast, IFN-α production by mDC is 10-fold lower in response to TBEV while they also produce high amounts of IL-6, IL-8, TNF-α and additionally IL-1β. In contrast to pDCs, mDCs showed impaired activation of surface molecule expression and supported productive TBEV replication. It is therefore tempting to speculate, that productively TBEV-infected mDCs disseminate the virus in the infected host but fail to prime and activate TBEV-specific T-cells properly.

Keywords: Dendritic Cells, tick-borne encephalitis virus, inflammatory cytokines, interferon-α

**Number I16**

## Immune and inflammatory response of calves after intrabronchial infection with *Chlamydophila psittaci*

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Objective: Although Chlamydophila (C.) infections are nearly ubiquitous in cattle, information about associated immune defence mechanisms is virtually absent for the bovine host. Thus, this ongoing study aimed at determining systemic and local immunological and inflammatory response evoked by an experimental chlamydia infection.
Methods: 24 calves were inoculated intrabronchially with 10e8 inclusion-forming units/calf of the bovine *C. psittaci* isolate DC15. The absolute numbers of blood leukocytes and immune cell subtypes were determined by microscopy and flow cytometry until 35 days post inoculation (dpi). Changes in mRNA expression levels of genes associated with inflammation and immune response were quantified from lung tissue samples and pulmonary lymph nodes using real-time RT-PCR.
Results: In blood, significantly increased absolute numbers of lymphocytes and granulocytes were accompanied by elevated numbers of CD4+, CD8+, CD25+ T cells at 2 dpi. Peak proportions of CD8+/WC1+ T cells and monocytes occurred between 7 and 35 dpi. In lung tissue, iNOS, RANTES, and IL-12 were transcribed at significantly higher rates after infection. Pulmonary lymph nodes of infected calves showed significantly enhanced expression of mRNA coding for iNOS, RANTES, TNF-alpha and IL18.
In conclusion, experimental exposure of the bovine respiratory tract to *C. psittaci* induced local and systemic reactions, as well as inflammatory responses.

# Poster Session – Infrastructure, Methods and Diagnostics

**Number D1**

## Microbiological and molecular detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in experimental infected calves

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Paratuberculosis is a chronic, not treatable intestinal inflammation on domestic and wild ruminants, which goes along with heavy economic losses for dairy farms. Additionally, the pathogenic agent *Mycobacterium avium* ssp. *paratuberculosis* has been suspected for years to be involved with the formation and/or maintenance of the human disease, known as Crohn's Disease.
In the scope of the innovation project „Frühdiagnostik von Infektionen mit MAP bei Rindern" ("Early diagnostics of infections with MAP on cattles"), six calves in each of two staggered experimental series were artificially infected with a MAP reference strain (ATCC BAA-968). In advance, a control group was stabled. Feces samples and biopsies of jejunal and ileocaecal lymph nodes from all calves were taken into consideration for the diagnostics. Cultural and molecular methods were done, using in parallel the TaqMan®-Real time PCR and the nested-PCR. So far obtained results of the microbiological and molecular analyses showed that an early detection of MAP in biopsies of lymph nodes of infected calves was possible, in some cases already on the 14$^{th}$ and 30$^{th}$ day post infection but surely on the 90$^{th}$ day.
In parallel, a field study on animals from farms with paratuberculosis issues is carried out, in order to evaluate the collected data from the experimental series.
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).

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*, calf, infection model, early diagnosis, lymph nodes

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**Number D2**

## Differential phenotyping of *Brucella* species

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**Background:** A commercial biotyping system testing the metabolization of various substrates by bacteria was used to determine if a set of phenotypic features will allow the identification of *Brucella* and differentiation of its closely related species and biovars.
**Methods:** A total of 191 different amines, amides, amino acids, other organic acids and heterocyclic and aromatic substrates, 191 different mono-, di-, tri- and polysaccharides and sugar derivates and 95 amino peptidase- and protease-reactions, 76 glycosidase-, phosphatase- and other esterase-reactions, and 17 classic reactions were tested with the 23 reference strains representing the currently known species and biovars of *Brucella* and with a collection of field isolates. Based on specific and stable reactions a 96-well "*Brucella* identification and typing" plate (Micronaut™, Merlin Diagnostika) was designed and re-tested in 113 *Brucella* isolates and clinically relevant bacteria.
**Results:** *Brucella* species and biovars revealed characteristic metabolic profiles and each strain showed an individual pattern. Due to their typical metabolic profiles a differentiation of *Brucella* isolates to the species level could be achieved. At the biovar level, *B. abortus* bv 4, 5, 7 and *B. suis* bv 1-5 could be discriminated with a specificity of 100% whereas *B. melitensis* isolates clustered in a very homogenous group.
**Conclusions:** The comprehensive testing of metabolic activity allows cluster analysis within the genus *Brucella*. The biotyping system developed may replace or at least complement time-consuming tube testing especially in case of atypical strains. An easy to handle identification software facilitates the applicability of the Micronaut™ system for microbiology laboratories.

Keywords: *Brucella*, biotyping, phenotyping, metabolism

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**Number D3**

## Fundamental investigations for functional epidemiology of *Giardia duodenalis* infections

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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* (petidyl-) arginine deiminase (ADI) that converts (peptidyl-) 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 and charting their enzymatic activities. Particularly, their activity patterns on substrates with likely pathophysiological relevance (e.g. fibrinogen, collagen) shall be determined. 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 that was cloned, recombinantly expressed and purified by affinity chromatography. Furthermore, enzyme activity

tests were adapted for colorimetric measurement of arginine to citrulline conversion.

Keywords: Giardia duodenalis, arginine deiminase, zoonosis, epidemiology

**Number D4**

## Recombinant γ Phage Lysin as a Substitute for the *Bacillus anthracis* γ Phage in Anthrax Diagnostics

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The lysis of cultures on agar plates by the γ phage is globally used and recommended by the CDC and WHO as a means to identify *Bacillus anthracis*. The γ phage is highly specific, and as of to date only a small number of *B. anthracis* isolates are known to be resistant, and only very few other bacilli are known to be lysed by this phage. The γ phage lysin (PlyG) has been suggested for use in anthrax diagnostics. The recombinant rPlyG was expressed in and purified from *E. coli*. We investigated the host specificity of rPlyG on 108 isolates of *B. anthracis* and 200 other bacilli. We also developed a fast and easy to conduct colorimetric assay for the detection of *B. anthracis* lysis. This assay has shown to deliver consistent and quantifiable data within hours and it is more sensitive than measuring cell lysis by OD600nm reduction. The suitability of this assay in routine detection of *B. anthracis* was evaluated. Host specificity of rPlyG was slightly diminished compared to the γ phage. However, unlike the γ phage, rPlyG is able to lyse encapsulated vegetative bacilli. It does not lyse spores or spores germinated for less than 90 min in vitro.

Keywords: phage, lysin, anthrax

**Number D5**

## Multi-diagnostic Serological Monitoring of Pig Herds at Slaughter Including Zoonotic Infections

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The paper reports on testing a multi-diagnostic, serological monitoring for identifying and quantifying health risks for humans carried by slaughter pigs into the food chain. The existing serological Salmonella control programme is mostly using meat juice as specimen for detecting Salmonella antibodies in slaughter pigs. The fact that the meat juice for the Salmonella monitoring is available, but not used for any other purpose has led to the idea to test the usability of meat juice for detecting also other infections in pigs. To achieve a high efficiency of taking and testing samples for antibodies, the samples were not only tested for zoonotic pathogens, but also for pathogens causing so-called production diseases of pigs.
The goal is to produce food safety risk and herd health profiles with estimations of the intra-herd prevalence for each pathogen. Creating a system of serological herd profiles for the most important infections, even on the basis of only limited sample sizes per herd, provides the opportunity for introducing benchmarking systems and for targeted decisions both for improving the food safety of pork and for improving the animal health of pig herds. As for the zoonotic infections, the authors tested the measuring of antibodies in meat juice against the following pathogens: Salmonella, Toxoplasma, Trichinella, Yersina.
The results of the presented study suggest that there are remarkable differences in the intra-herd prevalence of the tested pathogens.

**Number D6**

## Q-fever: diagnostic microbiology – what is necessary in an outbreak situation

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Q fever is an occupational disease affecting those with direct contact with mostly chronically infected animals, such as farmers or veterinarians. However, indirect contact with infected animals can result in outbreaks of Q fever like the one in Jena in 2005 where more than 300 patients living in the near of a grazing land for sheep developed acute Q fever with pneumonia. Only some of these patients consult the university hospital and allow us to evaluate diagnostic procedures of Q-fever in respect to the course of infection. Diagnosis of acute Q fever so far relies on the detection of specific antibodies. Delayed antibody generation and the need of experience to interpret the IFAT (reference method) often leads to misdiagnosis and underreporting of the disease. Thus a commercially available ELISA and a nested polymerase chain reaction from serum regarding time of serum collection were explored in the study. As PCR target the IS1111 sequence of the Coxiella genome was used. Serum samples of 22 patients with acute Q fever collected around the 5$^{th}$ day of illness (5.5 ± 2.8 days of illness - range 1 to 11 days) were included. Sensitivity of 30% by ELISA and 80% by IFAT (p=0.1) was found for the first five days of illness and 92% by ELISA and 83% by IFAT during the sixth and eleventh day. PCR revealed in 8 cases (36%) a positive result with six cases in the first five days of illness. This low rate requires the inclusion of respiratory samples in the PCR investigation.

Keywords: Q-fever, Coxiella burnetii, diagnostic sensitivity

**Number D7**

## Rapid screening of epidemiologically important *Salmonella enterica* subsp. *enterica* serovars using Whole-Cell MALDI-TOF mass spectrometry

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Currently, 2,579 different Salmonella serovars are described according to the Kauffmann–White scheme. They are routinely differentiated by serotyping, a rather time-consuming and expensive technique, which is based on the antigenic variability at lipopolysaccharide moieties (O antigens), flagellar proteins (H1 and H2 antigens), and capsular polysaccharides (Vi antigens). The aim of this study was to evaluate the potential of MALDI-TOF mass spectrometry for rapid screening and identification of the most prevalent *Salmonella enterica* subsp*. enterica* serovars based on specific sets of serovar-identifying biomarker ions. By analyzing 681 *Salmonella enterica* subsp*. enterica* strains representing 82 different serovars using MALDI-TOF mass spectrometry, several potentially serovar-identifying biomarker ions were selected. Based on a combination of genus-, species-, subspecies and serovar-identifying biomarker ions various *Salmonella enterica* subsp*. enterica* serovars, including serovars Enteritidis, Typhimurium/4,(5),12:i:-, Virchow, Infantis, Choleraesuis, Heidelberg and other prevalent serovars could be rapidly identified. By using a bioinformatic approach sequence variations corresponding to single or multiple amino acid exchanges in several biomarker proteins were identified supporting biomarker selection. Whole-Cell MALDI-TOF mass spectrometry could be a rapid means for the pre-screening of *S. enterica* subsp. *enterica* isolates to identify several prevalent serovars and to reduce sample numbers that have to be subsequently analysed using conventional techniques.

**Number D8**

## A pentaplexed quantitative real-time PCR assay for the simultaneous detection and quanti-fication of botulinum neurotoxin-producing clostridia in food and clinical samples

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Botulinum neurotoxins (BoNTs), produced by the anaerobic bacterium Clostridium botulinum, are divided into 7 serotypes (A–G) known to cause botulism in animals and man. We developed a multiplexed quantitative real-time PCR assay for the simultaneous detection of the human pathogenic C. botulinum serotypes A, B, E, and F. Based on the TaqMan chemistry, we used five individual primer/probe sets within one PCR reaction, combining both minor groove binder- and locked nucleic acid-containing probes. Each hydrolysis probe was individually labeled with distinguishable fluorochromes, thus enabling the discrimination between the serotypes A, B, E, and F. To avoid false-negative results we designed an internal amplification control (IAC), which was simultaneously amplified with the four target genes. Additionally, we developed six individual singleplex real-time PCR assays based on the TaqMan chemistry for the detection of the C. botulinum serotypes A, B, C, D, E, and F. Upon analysis of *C. botulinum* and non-*C. botulinum* strains, the singleplex and multiplex PCR assays showed an excellent specificity. Using spiked food samples we were able to detect between 10exp3 and 10exp4 CFU/ml, respectively. Furthermore, we were able to detect C. botulinum in samples from several cases of botulism in Germany. Overall, the pentaplexed assay showed high sensitivity and specificity and allowed for the simultaneous screening and differentiation of specimens for C. botulinum A, B, E, and F.

Keywords: Botulism, real-time PCR, internal amplification control, food, clinical samples

**Number D9**

## Comparison of automated nucleic acid preparation methods for zoonotic viruses in serum, ticks and rodent tissue

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Nucleic acid (NA) sample preparation remains a crucial step, for molecular diagnostics of zoonotic viruses and also scientific molecular applications. The quality of NA isolation can severely affect the analytical sensitivity, specifity and the dynamic range in diagnostic assays. Therefore, we compared a manual NA extraction Kit (Qiagen) with three NA isolation automation systems, i.e. the Roche Magnapure compact, the PSS Magtration 12GC and the Fuji QuickGene instrument.
As DNA-virus we used an orthopoxvirus (Vacciniavirus Elstree) and as RNA-virus a flavivirus (Usutuvirus) in serial dilutions. Four different virus matrices spiked with virus were investigated: cell culture medium, sera, mouse liver tissue and tick homogenates, respectively. Each preparation was set up and determined four-fold. The extracted NAs were quantified using a specific real-time PCR and RT-PCR on the Light cycler instrument. For the evaluation of the NA extraction method resulting values of Ct of specific target and internal controls were taken for statistical analyseswith SPSS software (e.g. ANOVA, linearity by linear regression, etc.).
An index was calculated for each virus, matrix and instrument based on the statistical result, also including a time-cost factor for each NA extraction method. Furthermore mobility properties, e.g. size and weight, of the systems for field investigation were evaluated.
Interestingly the results for the manual and automatic nucleic preparations were very different for the four matrices. We conclude that for an adequate preparation of viral DNA and RNA from different matrices the used NA isolation methods have to be compared and evaluated carefully.

Keywords: NA isolation, automation, poxvirus, usutuvirus

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**Number D10**

## Recombinant antigens facilitate determination of *Toxoplasma gondii* infection in man and animals

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Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. This parasite is one of the most successful protozoa which infects cats as primary hosts but can also infect other mammalians and birds. *T. gondii* exists in the stage of tachyzoites, bradyzoites and oocytes. The latter are only present in primary hosts. Each form is known to express stage-specific antigens.

We expressed twelve parasite antigens in *E. coli*. The obtained recombinant antigens were then applied to the format of a lineblot assay in order to investigate their ability as diagnostic markers. For this, human serum samples were analyzed for the presence of *T. gondii*-specific IgA, IgM and IgG antibodies. In addition, recombinant antigens were also tested on serum samples of diverse animals that were infected experimentally with either oocysts or tachyzoites of *T. gondii*.

The resulting titer kinetics of the respective animals showed antigen-specific differences that may allow drawing a conclusion of the way of infection. Furthermore, animal species-specific differences were observed for the immunogenicity of the recombinant antigens; e. g. rGRA1 seems not be detected by the humoral immunity of poultries. In contrast, all GRA antigens showed a potential as diagnostic markers for the presence of IgG antibodies in human serum samples. While most of these antigens seem not be useful for determination of the infection stage (acute versus chronic/latent infection), the C-terminus of rPX and rGRA6 offered properties as acute stage-specific markers on human as well on animal serum samples.

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

Keywords: Toxoplasmosis, recombinant antigens, diagnostic

**Number D11**

## External quality assurance exercises for the detection of high threat cacteria in the framework of a European network

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The prevalence of zoonotic bacteria with high virulence like *Francisella tularensis* is often unknown, or, in some cases, bacteria occur in modified forms like *Bacillus anthracis* sensu lato. Thus, a high diagnostic awareness for this group of bacteria is required for the rapid identification of single cases and epidemics in situations of both, natural and artificial biological threats.
Therefore, an EU funded project (EQADeBa 2007-204) on External Quality Assurance Exercises (EQAEs) for the detection of high threat bacteria, coordinated by the RKI has been launched. The project is focused on bacteria of potential bioterrorism risk causing anthrax, tularemia, plague, glanders, melioidosis, brucellosis, and Q-fever.
Two of three rounds of EQAEs examining samples of varying complexity have been carried out. The efficacy of improvement will be validated by a third round of EQAE.
The first round of exercises included 15 DNA samples from purified bacterial cultures and 15 more complex bacterial samples in different matrices. The diagnostic challenge was raised for the second EQAE when, in addition to 15 inactivated bacterial samples in complex matrices, 15 samples with living bacteria, partially mixed with "natural" contaminants, were applied.
In general, the diagnostic preparedness of the participating laboratories to identify the target bacteria was well developed although need for further improvement and the importance of continuous quality assurance were recognized.

Keywords: EQAE, high threat bacteria

**Number D12**

## Identification of Leptospira isolates by means of multilocus variable-number tandem repeat analysis

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Leptospirosis remains an important zoonosis in animals and man in Germany. The identification and classification of the causative agents is necessary for epidemiological studies and disease control. Up to date the laborious microagluttination test (MAT) remains the test of choice for classification of *Leptospira*. Molecular tools such as multilocus variable-number tandem repeat (VNTR) analysis (MLVA) have been developed.
In this study we applied the Pasteur-MLVA to test its functionality for classification of isolates from wild carriers, pigs and horses suffering from leptospirosis. Reference strains (31), isolates from rats (4), mice (11), pigs (6), and from horses with moon blindness (26) were included in the study. All strains were cultured at 30°C in EMJH liquid medium for 4 to 6 weeks. Clinical isolates were identified serologically by means of MAT using rabbit antisera against the 29 *Leptospira* reference antigens. For MLVA, the extracted DNA was amplified in presence of either primer pairs: VNTR-4, VNTR-7, VNTR-10, VNTR-Lb4 and VNTR-Lb5. A database of MLVA-fingerprints was built applying Bionumerics 5.1 software, and the isolates were classified according to their similarity to reference strains. Finally, MLVA results were compared to findings obtained by MAT.
First results show, that MLVA was useful for classification of *Leptospira* isolates up to the serogroup level. This cultivation-independent method can be an alternative for the rapid identification of *Leptospira*.

**Number D13**

## Diagnosis of an imported case of dog rabies in Bavaria, Germany

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On 27th of February 2010, an 11 week old dog was imported by a private owner from Bosnia-Herzegowina to Bavaria. The puppy was not accompanied by the bitch and rabies vaccination was incomplete. It had been bitten weeks before by an adult aggressive dog that was destroyed without further examination. The puppy did not develop symptoms of disease until March, when signs of lameness were observed. On 19th of March, the puppy was presented to a practitioner. During examination, the owner was bitten. Within the next 48 hours the dog´s condition rapidly worsened including progressive lameness, hyperesthesia, and salivation prior to euthanization.
Post mortem examination revealed no other than euthanasia-related abnormalities. Histology revealed a non-purulent polioencephalitis of brain-stem and cerebellum with mononuclear, perivascular infiltrates, proliferation of Glia cells, and abundant nerve cell necrosis. Cell necrosis without signs of inflammation was detected in Ammon´s horn and cortex. Only single Negri bodies were detected and restricted to Purkinje cells of the cerebellum. Smears from hippocampus, cerebellum, and medulla oblongata exhibited RABV-immuno-reactivity in IFT. RT-PCR with primers targeting sequences within the rabies virus nucleoprotein (N) gene amplified a 420 bp fragment showing 98% sequence homology to RABV X542 nucleoprotein (GenBank accession number AY03417). The infective agent could be cultivated in murine neuroblastoma-cells (Na 42/13).
This case report illustrates, that ongoing Rabies surveillance in wildlife, vaccination prophylaxis of domestic animals, and restrictions in movements of pets are still necessary and important measures to prevent the re-emerging of Rabies in countries with a Rabies-free status.

Keywords: Rabies, Lyssavirus, RABV

**Number D14**

## Construction of a selectable SARS-CoV replicon system with eGFP and *Metridia* luciferase reporter genes

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Severe acute respiratory syndrome coronavirus (SARS-CoV) was identified as the causative agent of the SARS outbreaks in 2002-2003. Based on an infectious cDNA clone of SARS-CoV strain Frankfurt-1, a non-infectious replicon system was established. Structural and accessory SARS-CoV genes that are not necessary for replication and/or transcription were replaced by sequences coding for the secreted *Metridia* luciferase and also for an eGFP-Zeocin fusion protein. Capped *in-vitro* transcribed replicon RNA was electroporated into BHK-J cells and replicon-containing cells were selected by addition of zeocin. Replication was determined by luciferase assay and detection of eGFP by both, immune fluorescence assay and immuno blotting.
High biosafety is required to study SARS-CoV pathogenesis with infectious virus. In the study of replicase-transcriptase complex proteins encoded by nonstructural protein (nsp) genes, such safety requirements can be circumvented by use of a replicon system. An advantage over similar systems is provided in our replicon by providing two reporter proteins, eGFP and *Metridia* luciferase. The latter is secreted in cell culture supernatant, facilitating high-troughput assays. The replicon can be used for drug screening (e.g. proteinase inhibitors), the identification of cellular interaction partners of nsps, as well as for highly efficient protein expression from several mRNAs at a time, for which we are currently developing it into a packaged gene delivery tool.

Keywords: SARS-CoV, replicon system, reporter genes

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**Number D15**

## Reverse genetic characterization of a Chikungunya virus that escapes serum neutralization in cell culture

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The outbreak of Chikungunya virus (CHIKV) in South Asia has generated new interest in vaccines and mechanisms of immune escape. For reverse genetics, a full-length infectious cDNA clone was generated from a virus isolated from serum of a patient returning from Mauritius in 2006. The resulting recombinant virus was termed rCHIKV. We used this tool to reconstruct and characterize a CHIKV variant selected during virus isolation containing a small deletion in the E2 protein (rCHIKV/E2del). The deletion resulted in the substitution of amino acid N245 to K and deletion of amino acids A246 and E247 in E2. rCHIKV and rCHIKV/E2del showed similar plaque phenotypes and growth in mammalian cells, but impaired replication in insect cells. Furthermore, rCHIKV/E2del showed no evidence of increased affinity to heparin, as suggested for similar mutants in the related Sindbis virus. In presence of CHIKV antibody-positive patient sera, rCHIKV/E2del was neutralized up to 40-fold less efficiently compared to rCHIKV in Vero cells. Still, it grew to wild type levels in suckling C57Bl/6 mice with specific replication in the stifle joint compartment. Taken together our data indicate that the rCHIKV/E2del variant represents a natural immune escape variant that was selected in cell culture during virus isolation.
rCHIKV/E2del showed no evidence of increased affinity to heparin, as suggested for similar mutants in the related Sindbis virus. Infection of suckling C57Bl/6 mice with rE2del yielded slightly increased titers in the joint/muscle compartment up to 4 days p.i. but reached wild type (rCHIKV) titers by 5 days p.i. In presence of CHIKV antibody-positive patient sera, rE2del was neutralized up to 40-fold less efficiently compared to rCHIKV in Vero cells.
We constructed an infectious cDNA clone of CHIKV and used this tool to reconstruct and characterize a CHIKV variant selected

during virus isolation containing a small deletion in the E2 protein (rCHIKV/E2del). The CHIKV E2 protein constitutes the major exposed part of the virus envelope.

Keywords: Chikungunya virus, infectious cDNA clone, immune escape

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**Number D16**

## Identification of *Clostridium chauvoei* and seven other pathogenic *Clostridium* species using a DNA microarray assay

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*Clostridium chauvoei* is the causative agent of blackleg in cattle, sheep and other ruminants and exhibits a high mortality rate among infected animals. Blackleg is very similar to malignant edema caused by *Clostridium septicum* and *Clostridium novyi*, other *Clostridium* species belonging to the gas edema complex, and *Bacillus anthracis*, making the unambiguously diagnosis of blackleg difficult using traditional microbiological techniques.
We have developed a DNA microarray assay for rapid and simultaneous identification of these pathogenic *Clostridium* species, including *C. chauvoei*, *C. septicum*, *C. carnis*, *C. perfringens*, *C. haemolyticum*, *C. novyi*, *C. histolyticum* and *C. sordellii*. The assay is based on species specific oligonucleotide capture probes immobilised on the microarray, which hybridise to biotinylated 23S rDNA PCR amplification products and subsequent detection by streptavidin horseradish peroxidase. Probe specificity was tested on 27 *Clostridium* species. DNA extractions from meat spiked with *C. chauvoei* allowed the detection of 343 - 425 CFU per gram meat. Furthermore, the microarray assay was successfully tested on DNA extractions from clinical samples.

Keywords: Clostridium chauvoei, gas gangrene, DNA microarray, 23S rDNA probes

**Number D17**

## Optimization and validation of DNA isolation methods for rapid detection of Mycobacterium avium ssp. paratuberculosis (MAP) in milk

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Since beginning of the 20th century a possible causal connection between Mycobacterium avium ssp. paratuberculosis (MAP), the causal agent of Paratuberculosis, and the aetiology of Crohn's disease of humans has been permanently discussed. In particular, food of animal origin arises as possible vector.
MAP's characterized by an extremely high ability to survive, in particular heat treatment. The cultural MAP diagnostics of milk and dairy products is complicated. On one hand because of the prolonged growthtime of MAP from several weeks to months and on the other hand by the fact that the MAP fraction which has survived the heat procedure is often not detectable by culture any more. Hence, molecular-based methods are necessary for a rapid and reliable MAP diagnostics.
In the present study series of experiments was applied for the optimization and validation of DNA extraction procedures for the detection of MAP by TaqMan®-Real Time-PCR. For this, artificially contaminated raw milk was prepared differently. In the first part DNA extraction is carried out comparative with two different commercial DNA extraction kits („High pure PCR
Template Kit", Roche, and „Maxwell 16® system", Promega). In the second part two different magnetic beads (Pathatrix, England and Chemicell, Berlin) are set before DNA extraction to assess their influence on the detection probability.
The aim of the study was to validate a procedure to capture MAP cells in raw milk which can be used for screening.

Keywords: Mycobacterium avium ssp. paratuberculosis, milk, PCR, magnetic beads

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**Number D 18**

## Development of a DNA microarray detecting and characterising *Clostridium botulinum* species

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Laboratory diagnostics for botulism makes high demands and is still challenging. Despite the common species name, botulinum neurotoxin producing Clostridia comprise more than one species. The mouse lethal assay is still considered the gold standard for the detection of botulism. Additionally, PCR is commonly performed to determine the toxin serotype.
For further characterisation and epidemiological questions, the toxin subtype and the physiological/ phylogenetic group of the strain in question are also interesting. For the acquisition of such an amount of information on a strain, a DNA microarray could be the method of choice. Three groups of genes were included as target genes on a DNA microarray addressing this diagnostic task: 1) Toxin genes of the 7 known serotypes and some of the subtypes, plus one marker gene for the presence of a neurotoxin gene cluster, 2) ubiquitous genes that show adequate sequence variations for the differentiation of the phylogenetic groups 3) Genes unique to the phylogenetic groups. Sequencing of the ubiquitous rnpB gene of all strains of our strain collection showed that the rnpB gene is suitable as target gene for group differentiation.

Keywords: *Clostridium botulinum,* DNA microarray, serotype, subtype, botulinum neurotoxin gene

**Number D19**

## The German Q fever network - Q-Fieber-Verbund

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Q fever is a disease caused by *Coxiella burnetii*. The primary reservoirs of the highly infectious agent are sheep, goats, and cattle. Due to its zoonotic character Q-fever infects also humans and causes a flu-like disease with fever, pneumonia, and sometimes endocarditis in chronic disease. In Germany single cases or small outbreaks are registered countywide every year. A recent outbreak in the Netherlands with more than 3,000 infected humans emphasizes the importance for Q fever research.
The German Q fever network (Q-Fieber-Verbund) was created to re-evaluate the epidemiological situation in Germany, investigate the aetiopathology of chronic patients, gain new insides into the pathogenicity of *C. burnetii* isolates, identify new reservoirs of the agent and improve diagnostic and typing methods.
Thus, the network focuses on serological studies to elucidate the epidemiological situation in Germany, examines human endocarditic biopsy material and types human *C. burnetii* isolates, will define new biomarkers for the investigation of host-pathogen interactions and will find new diagnostic markers. Furthermore, examination of ticks and new potentially reservoirs are important tasks to identify and evaluate risk biotops.

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

**Number D20**

## Deep sequencing analysis of *Chlamydophila psittaci*

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*Chlamydiaceae* cause a wide variety of health problems including pneumonia, trachoma, preventable blindness, and sexually transmitted diseases, spontaneous abortion, and infertility in animals and man. Many of these pathogens can infect more than one host and in some cases are zoonotic, notably *Chlamydophila (C.) psittaci* and *C. abortus* (Longbottom and Coulter, 2003).
The comparative genome analysis of *C. abortus* and other *Chlamydiaceae* provided a unique opportunity to investigate the microevolutionary events that generate genetic plasticity and contribute to the speciation in this important family of pathogens. (Thomson et al. , 2005) Based on these sequences, transcriptome and proteome studies are possible now.
In case of *C. psittaci*, the causative agent of psittacosis, there is no genomic sequence available. With high-throughput deep sequencing technologies, we obtained the genomic sequence and thus gained so the opportunity for functional analysis.
References: Thomson et al., 2005 - The Chlamydophila abortus genome sequence reveals an array of variable proteins that contribute to interspecies variation; Genome Res. 2005 May;15(5):629-40. Epub 2005 Apr 18;
Longbottom and Coulter, 2003 - Animal chlamydioses and zoonotic implications; J Comp Pathol. 2003 May;128(4):217-44.

Keywords: Next-generation sequencing, *Chlamydophila psittaci*, Genomics, Transcriptomics

**Number D21**

## Performance of various commercial assays for the detection of Toscana Virus (TOSV) antibodies

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**Background:** Sandfly fever virus (SFV) serotypes sandfly vever Naples virus (SFNV) and sandfly fever sicilian virus (SFSV) cause febrile diseases and Toscana virus (TOSV) is responsible for aseptic meningoencephalitis. Diagnosis and surveillance of TOSV depend heavily on virus serology.
**Objective:** Comperative evaluation of commercially-available serological assays for anti-TOSV antibodies compared to virus neutralization test (VNT).
**Study Design:** A group of 132 sera, previously observed to be reactive for IgG to various SFV serotypes by indirect immunofluorescence assay (IIFT) were reevaluated by IIFT, enzyme-linked immunosorbent assay (ELISA) and an immunoblot (IB) assay by Euroimmun, Diesse and Mikrogen. VNTs were performed for 99 sera using standard TOSV, SFSV and SFNV strains.
**Results:** A total of 31 samples (31.3%) out of 99 were reactive in VNT that comprise TOSV(15.2%), SFSV(11.1%) and SFNV(3.03%) serotypes. Two sera (2.02%) were reactive for more than one SFV serotype. For IgG detection, commercial assays displayed fair to moderate agreement. The agreement between VNT and the commercial assays were fair ($\kappa$:0.273 for ELISA, 0.208 for IIFT, 0.371 for IB). IIFT was identified as the most sensitive assay (100%) while ELISA displayed the highest specificity (88.1%). No significant correlations between assay results and VNT titres were observed. None of the IgM reactivities (9/132, 6.82%) could be confirmed via VNT.
**Conclusions:** Specificity/sensitivities of the commercially-available immunoassays vary significantly and require confirmation by more specific assays.

Keywords: Sandfly fever virus serodiagnostics, sandfly fever Sicilian virus, sandfly fever Naples virus, Toscana virus

**Number D22**

## Development of a flow-trough microarray based reverse transcriptase multiplex ligation-dependent probe amplification assay for the detection of European Bunyaviruses

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It is suspected that apart from tick-borne encephalitis virus (TBEV) several additional European Arboviruses such as the sandfly-borne Toscana virus (TOSV), sandfly fever Sicilian virus (SFSN) and sandfly fever Naples virus (SFNV), mosquito-borne Tahyna virus (TAHV), Inkoo virus (INKV), Batai virus (BATV) and tick-borne Uukuniemi virus (UUKV) cause aseptic meningo-encephalitis or febrile disease in Europe.
Currently, the microarray technology is developing rapidly and there are many efforts to apply it to infectious diseases diagnostics. In order to arrive at an assay system useful for high throughput analysis of samples from aseptic meningo-encephalitis cases we developed a combined Multiplex Ligation dependent Probe Ampilfication (MLPA) and flow through microarray assay for the detection of European Bunyaviruses. Our results show that this combined assay indeed is highly sensitive, and specific for the accurate detection of multiple viruses.

Keywords: Bunyaviruses, MLPA,flow through chip, diagnostic microarray

**Number D23**

## Rapid detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in milk

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*Mycobacterium avium* subsp. *paratuberculosis* is the causative agent of Johne's disease, a chronic enteritis of ruminants. Due to the possible association with Crohn's disease in humans, MAP has been widly discussed as a zoonotic agent. Milk may be contaminated directly within the udder or indirectly as a result of fecal contamination. Recently published studies have shown that low numbers of viable MAP may survive HTST pasteurization of milk.

The aim of the present study was to develop and validate a rapid fluorescence stain for the detection of viable MAP cells in milk. A combination of auramine orange (AO) staining and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) staining was used for the detection of MAP in spiked milk samples. AO identifies the total number of acid-fast bacteria, whereas CTC stains only viable bacteria by detection of bacterial respiratory activity.

In addition the *FASTPlaque*TB™ phage amplification assay combined with a MAP-specific real-time PCR was used for rapid detection of viable MAP cells in milk. With this assay the number of viable cells can be estimated within 48 h based on the count of plaques produced when D 29 mycobacteriophage-infected cells burst in a lawn of *M. smegmatis* indicator cells on agar plate.

First results of the comparison of CTC staining in combination with auramine orange, culture and the phage amplification assay are shown.

Investigations are assisted by the „Bundesministerium für Bildung und Forschung (BMBF)" (support code: 01KI0754).

The Dissertation is supported by a „Graduiertenstipendium der Justus-Liebig-Universität Gießen".

Keywords: Mycobacterium avium subsp. paratuberculosis (MAP), phage amplification assay, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), rapid detection, milk

**Number D24**

## Recombinant expression of alphavirus glycoproteins and evaluation of their potential use in diagnostic assays

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Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE) and Venezuelan Equine Encephalitis (VEE) are caused by RNA encoded, enveloped arboviruses that belong to the genus *Alphavirus.* These diseases are characterized by neurological symptoms or even death in humans, horses and other mammals and are found endemicly only in North- and South-America. Our aim was to develop an alternative test for the time-consuming and hazardous (BSL-3) virus cultivation needed for plaque-reduction-neutralisation-assays. For this purpose we have established standard immunoassay methods using inactivated viral antigen. Detection of antibodies against VEEV and EEEV was accomplished using a MAC-ELISA modified from the protocol of Sahu et.al. (1994), as well as an epitope-blocking-ELISA, first published by Wang et al. (2005). The functionality of these assays was evaluated with a panel of positive and negative horse sera. In order to become independent from virus cultivation, we have expressed the main immunogenic proteins, envelope protein E1 and E2, in a prokaryotic system. Here we present very promising comparative data on the use of the recombinant and of inactivated virus antigens for the detection of antibodies against equine encephalitis viruses.

Keywords: Alphavirus, ELISA, recombinant expression, envelope protein, equine encephalitis

**Number D25**

## Rodent-borne zoonoses - Bringing the lab to the mice

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Field investigations are frequently conducted to study focal outbreaks of rodent-borne zoonoses like Hantavirus infections or to gain insights into the distribution of a disease agent in the animal reservoir. However field work is usually limited to the catching of the rodents whereas dissection, sample preparation and microbiological analysis are mostly conducted in stationary laboratories distant from the field site and may be also influenced by sample transport conditions and times.
We present an alternative approach, using modular, rapidly deployable field lab equipment. A core capability of this equipment consists in utilising even very basic facilities within a short space of time for epizootological investigations. Several operational areas (mouse euthanasia, dissection, tissue sample preparation, real-time-PCR) can be created in such an area with the aid of various materials. To protect the operators, personal protective equipment, as well as a mobile glove box is used until possible pathogens have been safely inactivated.
The deployable field lab concept has been successfully applied in a Hantavirus monitoring project: Following a case of nephropathia epidemica acquired on a military training ground we are monitoring the affected area once a year since 2007. Using the deployable lab in connection with a novel pan-Hantavirus real-time RT-PCR, time needed for field work, sample analysis and risk assessment could be significantly reduced from several weeks to 5 days only.
This novel approach greatly enhances the speed of pathogen detection. The ability to monitor rodent-borne disease outbreaks within a short time period therefore allows more rapid recommendations for exposure prophylaxis and rodent control measures.

Keywords: Hantavirus, field investigation, real-time RT-PCR, deployable lab

# Poster Session – New and Re-Emerging Infectious Diseases

**Number N1**

## Pyrosequencing of Tribeč virus, a tick-transmitted Orbivirus of the Kemerovo serogroup

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Orbiviruses include pathogenic agents of man (Kemerovo virus, Tribeč virus), domestic animals (Bluetongue, African horse sickness), native animals (Epizootic haemorrhagic disease) and many other viruses not yet linked with disease. They are transmitted by midges, ticks, phlebotomine flies and mosquitoes and have genomes consisting of 10 segments of double-stranded RNA (dsRNA) coding for at least 10 viral proteins. Sequences are available for many of the insect-borne Orbiviruses, but to date sequence data are available only for three non-pathogenic tick-borne Orbiviruses: Broadhaven virus (partial), Sandy Bay Virus (partial), both members of the Great Island virus serogroup and St Croix River virus (complete genome). The complete genome sequence of tick-borne Tribeč virus, an Orbivirus of the Kemerovo serogroup that can cause diseases of the central nervous system, was determined by a combination of FLAC-sequencing and pyrosequencing. It represents the first complete genome sequence of a tick-transmitted human-pathogenic Orbivirus and can be used to develop diagnostic tools. The predicted outer-shell protein VP2 of Tribeč virus has only about half the size of VP2 proteins of insect-transmitted Orbiviruses. Therefore these new sequence data support previous observations indicating significant differences between insect- and tick-borne Orbiviruses. Genome comparisons with non-pathogenic Orbiviruses could help in determining the molecular reason for pathogenicity of Tribeč virus.

Keywords: Tribeč virus, pyrosequencing, Orbivirus, Kemerovo serogroup

**Number N2**

## Pyrosequencing of Kemerovo virus, a tick-transmitted Orbivirus found in Siberia

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Kemerovo virus is a tick-borne Orbivirus that was isolated from ticks and cerebrospinal fluid of two patients with encephalitis in western Siberia in 1962. It is the type species of the Kemerovo serogroup within the Orbivirus genus. The genome consists of 10 segments of double-stranded RNA (dsRNA). So far no sequences were available for Kemerovo virus. With a combination of FLAC-sequencing and pyrosequencing we were able to determine almost the complete genome sequence of tick-transmitted Kemerovo virus in one sequencing run. The predicted VP1 protein (RNA-dependent RNA polymerase) of Kemerovo virus shows 79 % homology to the VP1 protein of Tribeč virus, another Orbivirus of the Kemerovo serogroup that we were able to sequence and that can be found in Germany. The homology to other Orbivirus VP1 proteins is 38 to 49%. The predicted VP3(T2) protein of the inner shell shows 91% homology between Kemerovo and Tribeč virus and 77% homology to other tick-transmitted non-pathogenic Orbiviruses of the Great Island virus serogroup. The sequence variations of Orbivirus VP3(T2) proteins correlate with virus serogroup. Therefore our sequence data support the serological classification of Kemerovo virus and Tribeč virus into the Kemerovo serogroup of Orbiviruses, which is distinct from the Great Island virus serogroup.

Keywords: Kemerovo virus, pyrosequencing, Orbivirus

**Number N3**

## Pyrosequencing of Erve virus, a European Nairovirus distantly related to Crimean-Congo hemorrhagic fever virus

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Erve virus is a Nairovirus that is found in France, Germany, The Netherlands and Czech Republic. It is suspected to cause severe headache (thunderclap headache) and intracerebral hemorrhage. Erve virus shows antigenic similarity to Crimean-Congo hemorrhagic fever virus. The mode of transmission to humans (ticks or mosquitoes) is still unknown. Currently no standardized testing method for Erve virus exists and only a small partial sequence of the polymerase gene is available. We were able to sequence the complete genome of Erve virus via pyrosequencing. It consists of 3 segments of negativ-stranded ssRNA (L: 11.6 kb, M: 3.9 kb and S: 2.1 kb). Phylogenetic comparison of the amino acid sequence of the putative L-protein (RNA-dependent RNA Polymerase) revealed only 48% homology to available L-protein sequences of other Nairoviruses like Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus, Hazara virus, Kupe virus and Dugbe virus. Among themselves these Nairoviruses show 62 to 89 % homology in the L-protein sequences. Therefore Erve virus seems to represent a member of a new subgroup of Nairoviruses. The new sequence data can be used for the development of diagnostic methods and the identification of the natural vector.

Keywords: Erve virus, pyrosequencing, Nairovirus

**Number N4**

## Pyrosequencing of Jug Bogdanovac virus, a sandfly-transmitted Rhabdovirus from Serbia

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Jug Bogdanovac virus was isolated in 1983 from the sandfly *Phlebotomus perfiliewi* in Serbia. Antibodies against Jug Bogdanovac virus were found in humans and domestic animals. Antigenic relationship was shown to Chandipura virus from India and West Africa and Isfahan virus from Iran and Turkmenia, two Rhabdoviruses of the Vesicular stomatitis serogroup that are implicated in causing neurological diseases in humans. So far, no molecular data have been reported for Jug Bogdanovac virus. We were able to sequence the complete genome of Jug Bogdanovac virus via pyrosequencing. The non-segmented negative strand RNA genome is 11.2 kb in length and encodes 5 transcription units for the Nucleoprotein, Phosphoprotein, Matrixprotein, Glycoprotein and the RNA-dependent RNA-Polymerase in 3' to 5' sense. The polymerase of Jug Bogdanovac virus shows 60% homology to that of Chandipura virus, 59% homology to that of Isfahan virus and 57% homology to that of Vesicular stomatitis virus on amino acid level. The homology to the polymerase of Rabies virus is only 34%. Our molecular data therefore support the serological classification of Jug Bogdanovac virus into the Vesicular stomatitis serogroup of Rhabdoviruses. The new sequence data can be used for the development of diagnostic methods.

Keywords: Jug Bogdanovac virus, pyrosequencing, Rhabdovirus

**Number N5**

## Seroprevalence of antibodies against Rift Valley Fever Virus in Southwestern Tansania

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Rift Valley Fever (RVF) is an arbovirus disease which is transmitted by mosquitoes and by direct contact with viremic blood or tissue from infected ruminants. Its main distribution is Sub-Saharan Africa, but RVF virus shows a stong tendency of expansion to the north and was already detected in Egypt, Saudi-Arabia and Jemen. Although RVF virus occurs throughout Sub-Saharan Africa, in many regions of those countries the epidemiology of RVF is unknown.
We tested 1.233 sera from nine different regions in southwestern Tansania on IgG antibodies against RVF virus. In one region (Kyela) 150 sera from one region with a high prevalence of IgG antibodies was also tested for IgM antibodies against RVF virus. Sera were tested using a commercdially available biochip (Euroimmun, Lübeck, Germany). Screening was done with a dilution of 1:10.
Testing 1.233 sera from the Mbeya region in Southwestern Tansania RVF virus IgG antibodies were detected in one region below 500 m of altitude. Regions with higher altitudes (up to 1.700 m) exhibited low seroprevalence rates. In the area of Kyela an IgG seroprevalence rate of 24% was detected. All sera available from Kyela region were also tested against RVF virus IgM. In 150 sera of the region 13/150 sera reacted positive in IgM.
Our study for the first time show ongoing RVF virus transmission in the Kyela region in southwestern Tansania. Further studies have to elucidate the medical importance of this arbovirus disease in the region.

**Number N6**

## Hantavirus epidemic in Southwest Germany in 2010 – an epidemiologic and clinical analysis of laboratory-identified and hospitalized cases

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The European variant Puumala of Old World hantaviruses causes a mild form of hemorrhagic disease with renal impairment called nephropathia epidemica. Southwestern Germany is the main endemic area within Germany, with most cases recognized in the state Baden-Württemberg. In 2010, a strong increase in hantavirus infections was seen with already 1055 confirmed cases by the end of July.
In this observational study, we analysed all hospitalized cases between January and July 2010 at the University Hospital Heidelberg for clinical presentation, risk exposure and course of disease and compared our findings to the outbreak of 2007. Further, we collected epidemiological data from registered cases in cooperation with the local health office in Heidelberg. Our clinical cohort consisted of 50 patients (serologically confirmed by positive IgG and IgM) within an area of approximately 40 km and recognized a subset of cases from areas previously not considered a risk area for hantavirus infection. The ratio between men and women was 34 (68%) to 16 (32%), mean age was 36.9 years. 12% of all patients were children with a mean age of 15.5 years. A possible risk contact was inquired in 48%, with mainly low risk activities like dog walking or outdoor sports. Altogether, we found that cases during this year's hantavirus epidemic in the first half of 2010 in Southwestern Germany showed a comparable, though broad clinical spectrum of symptoms and a steep rise in hantavirus infections including previously non-endemic areas.

Keywords: hantavirus, nephropathia epidemica

**Number N7**

## Sandfly Fever Turkey Virus, a classical virological characterization of a Phlebovirus from an epidemic in Turkey 2008

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Due to the global warming the increase of viral tropical diseases receives more attention. The knowledge on Sandfly fever virus is quite sparse. We herein describe classical virological investigations of a new phlebovirus of the Sandfly Sicilian group which was isolated in 2008 during a Sandfly fever outbreak from a human patient from Turkey. The aim of the study was to characterize this new virus by classical virological methods: growth kinetics in Vero cells, cytopathic effects by different staining methods and morphogenesis by high pressure freezing electron microscopy. Virus titers ranged up to $10^{6,2}$ Tissue Culture Infections $Dose_{50}$/ml. After a continuous increase over 42 h the one step growth curve reached a plateau. Cytopathic effects were shown as restructuring of the cytoplasm going along with changes of nuclear formation 24 h p. i. and cells exhibiting a clear immunofluorescence-signal in the cytoplasm. Electron microscopy showed vesicles in the cytosol close to the Golgi stacks possibly transporting viral proteins from the Endoplasmatic Reticulum to the Golgi apparatus. Virus particles were also found within the Golgi stacks. Processing of the SFTV seem to take place via the Golgi apparatus. These results imply a direct effect of the viral intracellular processing on the cytopathology of infected cells. Further investigations will be performed to characterize the new phlebovirus.

Keywords: phlebovirus, electron microscopy, Sandfly fever

**Number N8**

## Interspecies transmission of animal Coronaviruses (CoV) - binding and infection studies in bat cells using soluble spike proteins and a pseudotype system

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The family of *Coronaviridae* comprises a large number of unsegmented RNA viruses that show a worldwide distribution and have an impact in public health and animal farming as they are able to infect humans (NL63) as well as animals like pigs (TGEV), cattle (bCoV), and poultry (IBV). There are attenuated viruses that are associated with the common cold but the appearance of the *severe acute respiratory syndrome* associated coronavirus (SARS) showed the potential risk for public health by an emerging coronavirus. By detection of viral RNA in bat fecal samples and sequence analysis it is now believed that bats serve as a natural reservoir for CoV.
The viral spike protein plays a crucial role for the establishment of an infection and we use different strategies to investigate the ability of an interspecies transmission: On the one hand we perform binding assays (IFA, FACS) on bat cell lines with soluble spike proteins lacking their membrane anchor. In a second approach we use pseudotyped vesicular stomatitis virus in which we incorporate different spike proteins and monitor their potential to mediate infection. In future studies we want to identify a possible cellular binding partner via virus overlay assay.

Keywords: bats, coronaviruses, SARS, binding, interspecies transmission

**Number N09**

## Characterization of Gouléako virus – a novel Bunyavirus isolated from West African *Culex nebulosus* mosquitoes

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Many members of the family *Bunyaviridae* are classified as emerging and re-emerging pathogens due to their recent invading of new habitats and increasing incidence in humans. However, according to their extremely high diversity sequence information for most members is lacking and classification is mainly done by serological methods. Furthermore, the establishment of molecular methods for bunyavirus detection is crucial for surveillance of arthropod populations and diagnostics in endemic areas where infections are mostly misclassified as malaria.
Here, we report the characterization of a novel bunyavirus by using a relatively simple approach to generate full genome sequences of unknown bunyaviruses. The virus was termed Gouléako virus (GOUV) and isolated during a surveillance campaign of arthropod-borne infections in rainforest edge habitats in West Africa. GOUV was detected in 28 mosquito pools (15.4%) of diverse habitats. Viral particles with bunyavirus-like morphology were detected by electron microscopy. The complete genome of S, M and L segments was sequenced, comprising 1087 nt, 3188 nt and 6355 nt, respectively. No ORFs encoding for the NSs and NSm proteins were detected. Phylogenetic analyses performed on all genome segments placed GOUV basal to Phleboviruses indicating that they share a common ancestor.
Further studies investigating if GOUV occurs in local vertebrates or the human population are needed to evaluate the zoonotic potential.

Keywords: Arbovirus, Bunyavirus, vector-borne disease, anthropogenic disturbance, mosquitoes

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**Number N10**

## Full genome sequencing and molecular characterization of a novel Bunyavirus from West African mosquitoes

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With more than 350 members, the *Bunyaviridae* is the largest family of RNA viruses and more than 60 viruses are responsible for mild to severe human diseases. Bunyaviruses are divided into five genera according to serological relationships and genome organization patterns. Knowledge on bunyaviruses circulating in Africa is sparse and molecular characterization is mostly lacking.
During a surveillance campaign for mosquito-borne infections a novel bunyavirus was isolated from West African mosquitoes. The virus was tentatively termed Herbert virus (HERBV) and detected in 28/432 mosquito pools (29%) consisting of different species of *Anopheles*, *Culex* and *Uranotaenia* mosquitoes. The tripartite genome was sequenced, comprising 1090 nt, 2684 nt and 7428 nt, respectively. No ORFs encoding for the NSs and NSm proteins were detected. The putative G1 protein seems to be trunctuated at the 3′ end. Furthermore potential cleavage and glycosylation sites and cysteine residues were identified. Phylogenetic analyses performed on all genome segments showed that HERBV is ancestral to Orthobunyaviruses on all segments and on the M segment as well ancestral to tospoviruses indicating possible recombination events. If HERBV has public health importance has to be determined.

Keywords: Arbovirus, Bunyavirus, emerging disease, mosquito, Africa

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**Number N11**

## Isolation of a sylvatic St. Louis encephalitis virus from *Culex nigripalpus* mosquitoes collected in Mexico

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Several arboviruses have extended their habitats and host ranges in recent years, causing epidemics in humans. The true diversity of some arboviruses in their original reservoirs, as well as the ecological conditions enabling them to leave their enzootic cycles remain unknown.
As part of a comprehensive study on the distribution of arboviruses and their vectors in different ecological habitats, 3.618 mosquitoes were caught in and around the Palenque National park in southeastern Mexico. To date 1.396 mosquitoes have been processed, including species classification, pooling (n=153 pools), and testing with generic PCR assays. Flaviviruses were detected in 63 pools, showing relationships to viruses typically limited to mosquitoes (62 pools), as well as to SLEV (one pool). Screening of individual mosquitoes confirmed the presence of SLEV in one *Culex nigripalpus* mosquito caught at the forest edge. Using primers for conserved regions a 903 nt fragment of the envelope gene and a 930 nt fragment of the NS5 gene have been amplified so far. Phylogenetic analyses in both genes indicated the tentatively named SLEV Palenque strain shares an old common ancestor with all presently known SLEV strains, differing in the E gene from all other strains by 1.7 - 3.4 % on amino acid and by 17 - 20 % on nucleotide level (p-distance). This nearly doubles the known diversity in SLEV and suggests sylvatic reservoirs may contain a larger spectrum of viruses than currently known from epidemic isolates.

Keywords: SLEV, Arbovirus, vector-borne disease, anthropogenic disturbance, mosquito

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**Number N12**

## Prevalence of orthopoxvirus antibodies in humans

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Recent reports of multiple zoonotic human cowpox virus (CPXV) infections transmitted directly from cats or pet/food rats resulted in discussions on an increasing health threat of the human population in Europe. CPXV is a rodent-borne orthopoxvirus with a broad host range and contains the largest and most complete genome of all poxviruses, including genomic parts with high homology to variola virus (smallpox). All members of the genus Orthopoxvirus, including CPXV and vaccinia virus (VACV) used for smallpox vaccination, are serologically cross-reactive and can therefore not be differentiated in serological studies.

A pilot seroprevalence study was initiated to evaluate the circulation of orthopoxviruses in different human population groups from Germany. Prevalence data were obtained from sera of a population-based randomized survey (n=1038) and a population with a supposedly higher risk (forest workers, n=465). In comparison, a randomised foreign human population from Western Africa (n=693) was analysed.

The proportion of seropositive samples in formerly VACV vaccinated persons versus non-vaccinated persons in all analysed groups will be presented. The results are used to discuss further extended seroprevalence studies in different human populations in Germany. Furthermore, methodological approaches are discussed for analysing changes of the serostatus over time in individuals of different populations to proof an increased incidence of circulating CPXV. Due to the fact that CPXV strains are genetically very heterogenous viruses, these data will help to perform a risk assessment about future CPXV infections in humans.

Keywords: poxvirus, cowpox virus, prevalence, seroprevalence

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**Number N13**

## Network „Rodent-borne pathogens“: Detection of a shrew-borne hantavirus in Germany

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The network "Rodent-borne pathogens" was initiated to get insights in the geographical distribution and molecular diversity of rodent-borne zoonotic pathogens, e.g. *Leptospira* spp., tick-borne encephalitis virus, cowpox virus and hantaviruses.
In Germany, at least three rodent-borne human pathogenic hantavirus species are circulating: *Puumala virus*, *Dobrava-Belgrade virus* and *Tula virus*. As the shrew-borne Seewis virus has previously been detected in *Sorex araneus* from Switzerland, the objective of our study was to prove the presence of shrew-borne hantaviruses in Germany.
Embedded in the network "Rodent-borne pathogens" a total of 197 insectivores were trapped during 2005-2009 in 10 different federal states of Germany. The animals were necropsied in a BSL3 containment laboratory. For hantavirus detection total RNA was extracted from lungs, reverse transcribed and amplified using primers targeting the polymerase encoding L genome segment.
We found four novel hantavirus L segment sequences from 59 initially investigated small mammals. These sequences originated from *Sorex araneus* trapped in three federal states of Germany, i.e. Thuringia, Hesse and Bavaria, are closely related to a Seewis virus sequence from Switzerland and another sequence from Saxony-Anhalt and suggest a geographical clustering. Further investigations are dedicated to characterize the molecular nature of this novel Seewis virus lineage in more detail and to prove its role for human infections.

**Number N14**

## Variability of the IFN-β promoter repressing activity of NSs proteins derived from field isolates of Rift Valley fever virus

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Variability of viral and host genetic factors may be involved in the pathogenesis of Rift Valley fever virus (RVFV) infections and may explain the wide range of clinical outcome in susceptible vertebrate hosts. The differences in virulence among RVFV isolates may be due to the interference with either the innate and/or the adaptive immunity. In this work the interferon-β antagonistic function of NSs of RVFV isolates from different sources (animals, humans, and insects) was assessed after cloning and sequencing the non-structural S segment gene (NSs). The NSs clones were monitored for their immune modulatory effects by analysing their ability to suppress the activation of the IFN-β promoter using a reporter assay system. Additionally, expression of NSs in Vero E6 cells was monitored by immuno-fluorescence staining. Two RVFV NSs proteins (derived from isolates R7 and R18) failed to inhibit IFN-β promoter activation whereas the remaining 24 showed efficient suppression of IFN-β promoter activity. Additionally R7-NSs and R18-Nss were unable to form nuclear filaments which are a typical feature of wild-type RVFV-NSs. Sequencing of R18-NSs revealed a large internal in-frame deletion identical to the mutation described for the naturally occurring RVFV mutant clone 13, which leads to a non-functional NSs-protein. Indeed, R18 was later identified as a RVFV clone 13 isolate. In contrast, R7-NSs contains a point mutation in the NSs gene, which results in the replacement of a leucine by proline. Interestingly, this unique point mutation has effects comparable to the large in-frame deletion of clone 13 NSs.

Keywords: Interferon-β, Rift Valley fever virus, NSs protein.

**Number N15**

## Clinical and laboratory findings of Sandfly Fever Turkey Virus outbreak in Ankara - running head: Sand fly fever virus outbreak in Ankara

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**Background:** Sandfly fever is an arthropod-borne disease, which is transmitted to humans by phlebotamine sandflies. In the summer of 2007, patients with high fever, severe myalgia, headache, photophobia, abdominal discomfort and nausea were presented to our department. All of them were from the same neighborhood "Mamak". Our results indicate that the patients had Sandfly fever. This report reviews the clinical and laboratory findings of the patients with Sandfly fever in our region.
**Methods:** A retrospective single-centre study was performed. Clinically suspected cases were defined on the basis of epidemiologic history and clinical and laboratory findings. The sera samples of the suspected patients were sent to Germany for diagnostic assistance. The clinical and laboratory findings of the patients were collected retrospectively.
**Results:** 51 patients (36 male, 15 female) were included in the study. All patients had fever at presentation. Headache, photophobia and conjunctivitis, myalgia, arthralgia, nausea, abdominal pain and anorexia were other common symptoms. Although the fever lasted only 3-5 days, complete recovery lasted up to 30 days. Leucopenia, thrombocytopenia and elevated serum aspartat-aminotransferase and alanine-aminotransferase levels were remarkable findings. The virus isolated from the sera samples were identified as "Sandfly Turkey Virus". The viral load was within the range of $3.19 \times 10^6$ to $2.79 \times 10^9$ viral RNA molecules detected /ml (mean: $5.78 \times 10^8$/ml, median: $1,14 \times 10^8$/ml).

**Conclusion:** The new type of sandfly virus is causing much severe elevated liver enzymes and thrombocytopenia, which was not underlined before in the literature.

Keywords: Sandfly fever Sicillian virus, clinical features

**Number N16**

## Lagos bat virus and its hosts - growth studies using fruit bat cell lines

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Rabies and rabies-related viruses are the causative agents for one of the most neglected zoonotic diseases. Like many other human pathogenic viruses, lyssaviruses circulate within different bat species. Only little is known about the molecular aspects, allowing bats to cope with such infections that lead to severe and mostly fatal diseases in humans. To gain insights into virus-host interactions, commonly used mammalian cell lines as well as recently established immortalized Eidolonhelvum and Rousettus aegyptiacus kidney cell lines were examined for susceptibility to Lagos batand Mokola virus infections. Direct fluorescent antibody test (dFAT) and real-time RT PCR confirmed susceptibility to Lagos bat virus. Highest titers where obtained in murine neuroblastoma cells (N2a), followed by E. helvum kidney cells (EidNi/3) and canine kidney cells (MDCK). Interestingly, one out of three subcloned Rousettus aegyptiacus kidney cell lines was just barely positive for viral antigens by dFAT and one subclone was completely negative even when infected at an MOI of 1 and incubated for 3 days. These studies revealed great discrepancies in susceptibilities of different bat cell lines to Lagos bat or Mokola virus. Our results are the basis for further examinations of the virus-host interaction of Lagos bat virus with Eidolon cells and for investigations into entry of lyssaviruses into Rousettus cell lines.

Keywords: Rabies, lyssaviruses, Lagos bat virus, fruit bats

# Poster Session – Pharmacology, Therapeutics and Resistance

**Number P1**

## Monitoring of antimicrobial resistance in zoonotic bacteria in Germany

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Resistance to antimicrobials in zoonotic bacteria is of special concern since these pathogens may compromise the effective treatment of infections in humans. To follow the occurrence of antimicrobial resistance Salmonella isolates from different sources are analysed regularly for risk assessment purposes.
Altogether, 33625 *Salmonella* isolates originating from feedingstuff, animals, food and environmental sources available from the years 2000 to 2008 were tested by using the microdilution method. The quantitative data were interpreted using harmonised epidemiological cut-off values.
Resistance to antimicrobials was commonly found among *Salmonella* isolates from all sources in Germany. Resistance to the commonly used antimicrobials of sulphonamide, tetracycline, ampicillin and some aminoglycosides were frequently observed. Resistance to ciprofloxacin and nalidixic acid were 7.2 % and 7.7 % respectively. Resistance to cephalosporins was observed in 0.4 % to 1.1 % of the isolates. The proportion of resistant isolates varied between the sources and *Salmonella* serovars considerably.
The observed ciprofloxacin resistance levels in *Salmonella* isolates, which are markedly high in certain populations, are of concern since fluoroquinolones are critically important antimicrobials in human medicine. Resistance to third generation cephalosporins, another critically important antimicrobial group, observed in some of the *Salmonella* isolates tested needs close monitoring and assessment.

Keywords: *Salmonella* spp.; antimicrobial resistance; epidemiological cut-off values; zoonotic agents; risk assessment

**Number P2**

## LaMRSA in conventional and organic pig herds in Germany

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The occurrence of laMRSA (livestock-associated Methicillin-resistant S. aureus) in pigs in conventional herds has been reported in several countries with often a very high intra-herd prevalence. Little is known about the frequency of laMRSA in organic pig herds. Our investigations on the occurrence of MRSA in different pig populations with different husbandry systems were carried out by the culturing nasal swabs and dust samples from conventional and organic pig herds using the same sampling strategy in both husbandry systems.
Our study showed that the laMRSA load (both in nasal swabs and in dust samples) in conventional husbandry systems turned out to be considerably higher than in organic husbandry systems. Possible reasons for the higher MRSA detection rate in conventional than in organic pig herds could be the circulation of MRSA through the more frequent animal movements between conventional herds than between organic herds and conventional herds, the higher animal density in conventional herds, and the commonly more frequent use of antibiotics in conventional herds. However, the differences in the MRSA frequency found in our study do not justify a direct link between the occurrence of MRSA and the actual use of antibiotics at a specific farm, since the spread of MRSA between conventional herds and the lack of this spread to organic pigs can explain the differences as well. More detailed research into the risk factors of MRSA is presently carried out.

**Number P3**

## RESET: ESBL and (fluoro)quinolone resistance in *Enterobactericeae*

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In 2009, the Federal Ministry of Education and Research announced financial support for research consortiums of antibiotic resistances. The proposal "RESET: ESBL and (fluoro)quinolone resistance in *Enterobactericeae*" was accepted for a three-year-period and will take up work in autumn 2010.
RESET includes studies on the dissemination of resistance properties of *E. coli* and *S. enterica* from humans, animals and the environment. The resistance to ß-lactams by Extended-Spectrum Beta-Lactamases (ESBLs) and to (fluoro)quinolones will be in the focus. The researchers aim at assessing the impact of different origins, transmission routes and pathogen attributes on the risk for humans.
Existing data and isolates from studies and surveillance activities will be completed and supplemented by epidemiological studies (humans, animals, food) and diagnostic investigation which may have an impact on human health.
This epidemiological information (distribution, prevalences) will be accompanied by molecular-based studies as well as typing and characterisation of strains and also by pharmacological investigations.
Finally, a risk assessment concept will be generated based on the available knowledge which will be a substantial contribution to DART ("Deutsche Antibiotika-Resistenzstrategie").
This consortium includes researchers from human and veterinary medicine, basic science and application-oriented research, microbiology and epidemiology, universities and government research centers.
Members of the Consortium: Charité, Federal Institute for Risk Assessment, Freie Universität Berlin, Friedrich-Loeffler-Institut
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Keywords: consortium, Salmonella enterica, Escherichia coli

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# Poster Session – Wildlife Zoonoses

**Number W1**

# Wild boars as reservoir for *Streptococcus suis* and leptospira in Germany

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Wild boars constitute an important hunting bag in Germany. Case reports of *Streptococcus suis* meningitis in hunters suggest transmission of *S. suis* through butchering of wild boars. Furthermore, wild boars in Berlin might have been involved in a case of human leptospirosis. The working hypothesis of our studies was that wild boars in Germany form a reservoir for leptospira and *S. suis*. Therefore, different tissue samples were collected from wild boars and screened for these pathogens. A total of 244 *S. suis* isolates from tonsils of wild boars were differentiated with respect to virulence-associated genes and AFLP. Results demonstrated that at least 10% of the investigated wild boars carried potentially human pathogenic *S. suis* strains as these strains belonged to the *epf*+ *mrp*+ *sly*+ *cps*2+ genotype and formed a homogenous cluster with invasive human and porcine strains. DNA from leptospira was detected with lipL41-PCR, lipL32- and lipL45-nested PCR in two samples taken from the urinary tract of two wild boars. Furthermore, microscopic agglutination test indicated positive antibody titers against leptospira in 85% of the serum samples (n = 65) with high prevalences against Bratislava (83%), Copenhageni (48%) and Grippotyphosa (20%). In conclusion, wild boars in Germany were found to be frequent carriers of virulent *S. suis* strains and might also be infected with leptospira. Wild boar hunters should be investigated with respect to these important zoonotic agents.

Keywords: boars, Streptococcus suis, leptospira, MAT

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**Number W2**

# Distribution and Evolution of genotypes of *Bacillus anthracis* in the wildlife of the Etosha National Park and their correlation to outbreaks in farm animals

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Anthrax is an endemic disease in Namibia with annual outbreaks occurring in the Etosha National Park (ENP) and sporadic outbreaks occurring in other private farms and in livestock animals throughout the country. More than 400 isolates of *B. anthracis* from carcasses and environmental samples, mainly collected in the ENP, were genotyped using a 31-marker MLVA, 12 SNP markers, and 4 SNR markers. The MLVA data were used for developing a consistent picture of the temporal and spatial distribution of genotypes (GTs) of *B. anthracis,* based on a UPGMA cluster analysis and the geographical mapping of the isolates. A minimum spanning tree, representing ENP isolates from between 1988 and 2010 shows a probable model for the evolution of GTs within the park. While 36 GTs were found in MLVA, all isolates from Namibia belonged to the same SNP group. Sub-typing of MLVA clusters by the SNR analysis allowed an even deeper understanding of the possible spread of the pathogen over time. Isolates from private game parks were closely related to the GTs in ENP, while outbreaks in livestock were mostly represented by different GTs from ENP.

Keywords: anthrax, genotyping, MLVA

**Number W3**

## A proposed natural transmission cycle of *Rickettsia helvetica* in Central Europe

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*Rickettsia (R.) helvetica* is the most common rickettsial species in Germany and is transmitted by *Ixodes* species. Its natural tranmsission cycle is unknown. We introduce a model of the natural transmsission cycle of *R. helvetica* deriving from our results of studies on ticks, rodents and ectoparasites for rickettsiae during the last years.
In PCR minimal infection rates of non-engorged tick larvae of 5 to 7% indicate that transovarial transmission plays a major role for maintaining a basic transmission cycle in the tick population. The percentage of positive ticks seems not to increase after moulting into nymphs. These data indicate that the main hosts for blood-sucking larvae, small wild rodents may not play a role as amplifying hosts for *R. helvetica*. This assumption is strengthened by low seroprevalence rates of *Apodemus flavicollis* and *Myodes glareolus* in Baden-Württemberg. After moulting from nymphs to adult ticks a significant increase of minimal infection rates from 5% up to 10 to 15% was detected. By chance, *R. helvetica* had been detected in two larvae of throat flies of a roe deer. This detection is interpreted as a strong indication that the roe deer developed rickettsiaemia with *R. helvetica* during the blood meal of these larvae from which rickettsiae wer eingested by the larvae.
We propose that *R. helvetica* in nature is maintained basically by transovarial transmission and that roe deer and perhaps other large game animals may play a role as amplifying hosts.

**Number W4**

## Close relationship between murine atypical enteropathogenic *E. coli* (aEPEC) and human aEPEC: are rodent aEPEC zoonotic pathogens?

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We conducted a study to define a possible reservoir function of wild living rodents for Shiga-toxin-producing *E. coli* (STEC) or enteropathogenic *E. coli* (EPEC). A total of 180 *E. coli* (12.8%) strains were isolated from 1.400 fecal specimens of wild living rodents. All isolates were screened for virulence genes *stx* (Shiga toxin), *eae* and *escV*, (markers for the Locus of Enterocyte Effacement (LEE)). None of the strains harboured *stx* genes, while 16 (8.8%) were characterized as atypical EPEC (aEPEC; *eae*+, *bfp*-, *stx*-).

Serotyping of rodent isolates revealed six strains sharing On.t:H6, five strains On.t.:NM, two strains O:rough:H-, and one strain each being O179:H31, O167:H and O26:H- respectively. In contrast to serotyping, all strains could be typed by Multilocus Sequence Typing (MLST), allowing the assignment of six strains to sequence type 28 (ST28), eight to ST1094, and one each to ST1092 and ST1104.

As this is the first report of aEPEC in wild rodents, we compared these strains with eight human aEPEC strains of highly related sequence types (ST), which had been isolated from patients suffering from diarrhea. Therefore, the rodent and human strains were further tested for virulence-associated genes, i.e., *astA, cdt, subAB* (toxins*), iha, paa,lpfA* (adhesins), for genes identifying the genomic O Island (OI) 122 (*pagC, sen, efa1*, *nleB*, *ent* ), *feoB, fyuA*, *irp2* (ironaquisition) and for insertion sites typical for shiga toxinogenic lambdoid phages as well as for clonal relatedness.

Results of serotyping and MLST of the human strains detected the same types, Ont:H6 and ST28, in three strains. None of the

aEPEC harboured genes for the genomic OI 122. Interestingly, in all human and rodent strains potential lambdoid phage insertion sites were intact, envisioning the possibility that these strains can be transduced by such phages.
These similarities between rodent aEPEC and human aEPEC show that wild rodents may be a source of infection of diarrheagenic aEPEC. Further studies are needed to deepen our knowledge on the epidemiology of aEPEC and to functionally characterize phage transduction conditions for rodent aEPEC.

**Number W5**

## Characterization and genome sequence of *B. cereus* biovar *anthracis*

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Untypical *B. anthracis*-like bacteria were isolated from wild great apes that had died of an anthrax-like disease in rain forest areas of Côte d´Ivoire (isolates CI) and Cameroon (isolates CA). In contrast to *B. anthracis* (Ba), the bacteria are motile, resistant to the diagnostic gamma phage, and CA isolates are also resistant to penicillin. Sequencing of the genome of one CI isolate revealed the presence of both the toxin (pXO1) and the capsule plasmid (pXO2) with almost 100% identity to the *B. anthracis* virulence plasmids. Regulation of toxin and capsule synthesis is comparable to Ba. The chromosome differs from that of typical *B. anthracis* strains by a functional flagella gene cluster and the lack of four prophage regions.

The gene for PlcR which controls expression of many virulence genes in the *B. cereus* group does not contain the nonsense mutation like in Ba but a different mutation, and expression analyses indicated that like in *B. anthracis*, the regulator PlcR is not functional in the CI and CA isolates.

In addition, the chromosome of the CI strain is characterized by several insertions up to 22 kb in size. Most of these regions are specific to the CI and CA isolates and can be used for specific detection of these particular bacteria. For the first time we describe bacteria that cause anthrax and combine chromosomal properties of *B. cereus* with both virulence plasmids of *B. anthracis*. Therefore, we suggest to designate these isolates as "*B. cereus* biovar *anthracis*".

Keywords: B. anthracis, B. cereus, virulence plasmids

**Number W6**

## Adenovirus with bat origin: possible intra-species transmission

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In 2008, a novel bat adenovirus (bat AdV-2 PPV1) has been isolated from free-ranging vespertilionid bats (Pipistrellus pipistrellus) after inoculation of vertebrate cell cultures with organ tissue material. The new virus was detected in 5 out of 210 examined bats from southern Germany. By using molecular biologic methods the tissue tropism of bat AdV-2 was investigated in all infected bats. Of all tested organs, the highest DNA copy numbers of bat AdV-2 were detected in the intestine, which suggests a correlation with a gastrointestinal disease. When analysing other animal parameters, the detection of this chiropteran virus could be connected with its transmission between individual bats living in close proximity to other bats.
After sequencing of the new bat AdV-2 by 454 technology, the whole genome of 31 kb was further analysed. The allocation of open reading frames was conducted and many conserved domains have been identified. Also, mapping of the whole genome, restriction map and analysis of the codon usage was performed. The phylogenetic analysis of whole genome, hexon-protein, fiberprotein and polymerase revealed a close relationship to canine adenoviruses.
Although adenoviruses are generally strong species specific, doubts have been raised for canine adenoviruses. Canine adenoviruses are unusually high pathogenic for dogs and have unexpectedly been isolated also from other carnivores like bears, wolfs and sea lions without showing any sequence difference or host adaption. Since other zoonotic viruses are known to have a bat origin, the hypothesis will be discussed that canine AdV may originate from some ancient bat AdV.

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**Number W7**

## Free-ranging European bats – a reservoir of zoonotic bacteria?

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Many studies have shown that wildlife can act as a reservoir of several infections agents that might affect humans, livestock and companion animals. In particular, the importance of chiropteran species as potential vectors of viral diseases has received growing recognition, especially in regard to human health. Meanwhile, investigations regarding bacterial pathogens in free-ranging bats, their impact on the individual host and a possible zoonotic importance are rare to nonexistent. Thus, we conducted a broad bacteriological and histo-pathological study in about 300 deceased free-ranging bats of 18 species found in Germany. The bat carcasses originated from four different geographic regions and were provided by bat researchers and bat protectionists. A total of 27 bacterial genera were isolated from bats including several bacterial species (e.g. *Yersinia* ssp., *Salmonella* ssp., *Staphylococcus aureus*) which are known as primary cause of diseases in humans and other animals. We found that nine bacterial species were clearly associated with inflammatory histo-pathologic changes and at least 16 % of all bats had died due to bacterial infection. Here, we provide an overview of bacteriological results from free-ranging vespertilionid bats. Molecular-biological investigations are in progress to identify bacterial genera in bats undetectable by routine culture methods to their relevance for animal and human health.

Keywords: chiroptera, infectious agents, pathology, wildlife

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**Number W8**

## Tick-borne encephalitis virus antibody prevalence in roe deer (*Capreolus capreolus*) sera

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In order to identify variables associated with the presence of tick-borne encephalitis virus, we conducted a serological survey of roe deer (*Capreolus capreolus*) in three forest districts of southern Hesse, Germany. Overall, 24/105 (22.9%) of the tested sera were positive (≥1:10 in the plaque reduction neutralisation test). Using a logistic regression model, we found that spatial autocorrelation (entered as factor "forest district"), indexed roe deer density (positive correlation), hind foot length of the tested roe deer (positive correlation) and infestation with female *Ixodes* spp. ticks (negative correlation) predicted the probability of TBE antibodies in roe deer sera. Temperature increase during spring time and host sex were rejected as explanatory variables.
Approximately, TBE seroprevalence reflected TBE incidence in humans in the counties in which we sampled the roe deer sera. However, we found considerable differences in seroprevalence (50.0% vs. 17.6%) between two forest districts located essentially in the same county. In order to produce appropriate TBE risk maps and to identify environmental and ecological correlates of TBEV circulation, we suggest downscaling the resolution (from county level to forest patch level) at which TBE infections are recorded.

**Number W9**

## Prevalence estimation of *Echinococcus multilocularis* in raccoon dogs (Nyctereutes procyonoides) in northern Brandenburg, Germany

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The human alveolar echinococcosis, caused by the larval stage of the small fox tapeworm *Echinococcus multilocularis*, is a lethal zoonotic infection with an incubation period in mean of 10 years. This 2-4 mm sized parasite, living in the small intestines of carnivores, is widely distributed in the Northern Hemisphere. Its main definitive host in Germany and other European countries is the red fox *Vulpes vulpes,* a most widespread and abundant canid species. In the federal states of Germany, studies on the occurance of *Echinococcus multilocularis* differ according to sample sizes, tested animal species and geographic distribution of the samples. In Brandenburg a yearly statewide monitoring of red foxes has taken place since 1991 and starting in 2000 the raccoon dog *Nyctereutes procyonoides* has been included in this investigation. The first evidence of *Echinococcus multilocularis* in two male raccoon dogs (*Nyctereutes procyonoides*) in northern Brandenburg, was provided by Thiess et al. in the year 2001. The raccoon dog is a medium-sized canid, originally distributed in East-Asia and its presence in Germany is proofed since the early 1960-ies.
Data on echinococcosis consist of the number of diagnosed positive and negative results directly linked in a Geographic Information System (GIS) to the corresponding communities. Due to the lack of samples and that positive cases did not appear in southern counties in raccoon dogs, evaluation was based on the summarized data of the years 2000 to 2008 only from five northern counties of Brandenburg. For estimation of prevalences we applied the Beta-binomial- model.

Keywords: raccoon dog, *Echinococcus multilocularis*, invasive canid, Brandenburg

**Number W10**

## Detection and characterization of a new putative Reovirus

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In the last years bats have been repeatedly reported to be hosts and transmitters of important zoonotic pathogens like Filo- and Henipaviruses and the SARS-like Coronavirus. Also several new Reoviruses originating from different bat species have been described, one of them associated with respiratory symptoms in humans. In order to investigate viruses of zoonotic potential from tropical rainforests, we collected bat samples in the Taï National Park in Côte d'Ivoire in November and December 2006. After inoculation of blood samples on a new fruit bat cell line, a novel virus has been isolated from lesser free tailed-bats (Chaerephon aloysiisabaudiae). Supernatant of cell cultures showing a cytopathogenic effect were subjected to electron microscopy and sequence independent PCR followed by sequencing. Electron microscopy gave first hints towards the presence of Reovirus-like particles. The obtained sequences showed up to 72 % similarity (amino acid level) with different segments of two Reoviruses (genus Coltivirus), Eyach virus and Colorado tick fever virus. The latter represents the most important tick-borne pathogen in the United States and is responsible for human febrile disease. It remains to be elucidated whether the new virus has an impact as a pathogen for humans and other primates.

Keywords: bats, Reovirus, Coltivirus

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