German Research Platform for Zoonoses

Germany 2014

Joint Conference:

German Symposium on Zoonoses Research 2014

and

7th International Conference on Emerging Zoonoses

Berlin, Germany

October 16-17, 2014

PROGRAM

Programme at a Glance

	Thursday, October 16, 2014
8:00	**REGISTRATION OPENS** **(POSTER MOUNTING) REGISTRATION OPENS**
8:30	
9:00	
9:30	
10:00	**PLENARY SESSION 1** **Ballroom**
10:30	
11:00	
11:30	
12:00	LUNCH
12:30	
13:00	POSTER VIEWING SESSION
13:30	
14:00	SESSION 1: (NEW AND RE-) EMERGING ZOONOTIC DISEASES PART A Ballroom / SESSION 2: ECOLOGY OF EMERGING ZOONOSES Room Steglitz / SESSION 3: EPIDEMIOLOGY, MODELING AND PREDICTION Room Zehlendorf
14:30	
15:00	
15:30	COFFEE BREAK AND POSTER VIEWING
16:00	**AWARD OF THE GERMAN ACADEMY OF VETERINARY HEALTH**
16:30	**CURRENT EBOLA VIRUS OUTBREAK IN WEST AFRICA** **Ballroom**
17:00	
17:30	**GENERAL ASSEMBLY NATIONAL RESEARCH PLATFORM FOR ZOONOSES (IN GERMAN)** **Room Zehlendorf**
18:00	
18:30	
19:00	
19:30	
	Friday, October 17, 2014
08:00	**YOUNG SCIENTISTS BREAKFAST**
08:30	
09:00	SESSION 4: (NEW AND RE-) EMERGING ZOONOTIC DISEASES PART B Ballroom / SESSION 5: NOVEL METHODS AND DIAGNOSTICS Room Steglitz / SESSION 6: PATHOGEN-CELL INTERACTION AND IMMUNITY Room Zehlendorf
09:30	
10:00	
10:30	COFFEE BREAK AND POSTER VIEWING
11:00	SESSION 7: RESERVOIRS OF ZOONOSES Room Steglitz / SESSION 8: RISK ASSESSMENT & PUBLIC HEALTH Ballroom / SESSION 9: VARIOUS TOPICS Room Zehlendorf
11:30	
12:00	
12:30	LUNCH
13:00	
13:30	POSTER VIEWING SESSION
14:00	
14:30	**PLENARY SESSION 2** **Ballroom**
15:00	
15:30	
16:00	**POSTER AWARDS AND CLOSING CEREMONY** **Ballroom**

Table of Contents

Welcome Address of the German Research Platform for Zoonoses

Dear colleagues,

We are delighted to welcome you to the 2014 Zoonoses Symposium. This year, the German Research Platform for Zoonoses has planned the *German Symposium on Zoonoses Research* with its partners from the USA – Heinz Feldmann and Juergen Richt – to coincide with the *7th International Conference on Emerging Zoonoses*.

Zoonoses are a global challenge – whether they are new or existing diseases, whether they are viruses, bacteria, parasites or prions, and whether transmitted directly or through vectors. The current outbreaks of MERS and Ebola underline the huge impact zoonoses can have on humanity, with consequences that go far beyond the obvious health concerns. Well-founded research on zoonoses can make a valuable contribution to a world where people and animals can live long and healthy lives.

We have created an agenda that we hope appeals to everyone. This includes interesting keynote speeches and parallel sessions covering a broad spectrum of topics. Zoonotic research encompasses a wide range of fields. And we would like to particularly highlight the keynotes on Thursday morning and Friday afternoon and the Ebola session that has been added in light of current events. Dengue fever, MERS, Ebola and *Yersinia enterocolitica* are examples of both new and existing zoonoses that underline the challenges facing researchers in this field, both in Germany and around the world.

Once again, our Young Scientists Breakfast will take place on Friday morning. Younger researchers are invited to take advantage of this relaxed setting to talk with experienced colleagues about possible career paths.

The conference is designed as a platform for exchanging up-to-date knowledge, meeting new partners and intensifying existing partnerships that span scientific disciplines and geographical boundaries. Let's join forces and bring the *One Health* idea to life.

We would like to take this opportunity to thank everyone that submitted abstracts, and prepared posters and presentations, some of whom have travelled a great distance to join us. All of you are making a positive contribution to the success of this conference.

Welcome Address of the German Research Platform for Zoonoses

We hope you enjoy the *Joint Conference – German Symposium on Zoonoses Research 2014 and 7th International Conference on Emerging Zoonoses* and benefit from the insights you gain.

Stephan Ludwig
(Muenster, Germany)

Martin H. Groschup
(Greifswald – Isle of Riems, Germany)

Sebastian C. Semler
(Berlin, Germany)

Directors of the German Research Platform for Zoonoses

Welcome Message of the International Zoonoses Conferences

Dear colleagues,

It gives us a great pleasure to welcome you to Berlin for the Joint Conference: German Symposium on Zoonoses Research 2014 and the 7th International Conference on Emerging Zoonoses.

The Conference builds on five successful German Symposia on Zoonoses Research and six successful International Conferences on Emerging Zoonoses, each of which has always provided an interdisciplinary forum for physicians, veterinarians, epidemiologists, immunologists, virologists, microbiologists, public health experts and others concerned with the ever increasing problems associated with the transmission of infectious diseases from animals to humans. Recent events obviously show the necessity and importance of international and interdisciplinary networks.

We hope the merged meeting will be a good opportunity to create synergies and will broaden networking and scientific exchanges in the zoonoses research community world-wide.

We welcome you to enjoy Berlin and its sites and wish you all an interesting, fruitful and enjoyable conference.

Heinz Feldmann Jürgen A. Richt

Co-chairs of the International Zoonoses Conferences

Introduction of the federal government to the German Symposium on Zoonoses Research 2014 and 7th International Conference on Emerging Zoonoses

Zooneses are a constant threat to humans and animals. The danger is latent constantly present and each new outbreak reminds us - science, politics, but also the public - of the severity of infectious diseases. The fight against infectious diseases is not yet won, as many believed years ago. The current Ebola outbreak in West Africa proves how dangerous infectious diseases are and how difficult it is to control them. Contractible diseases still range among the most common causes of death worldwide. According to WHO and OIE, over 60 % of emerging infectious diseases are zoonoses.

The potential spread of infectious diseases increases dramatically in our highly mobile time. As a matter of fact, we could never travel from one continent to another as fast as we can today. Never before could animals, goods and food be transported and traded as quickly as now. Due to globalization, many zoonotic pathogens become highly dangerous.

Especially the citizens of poorer country suffer, since effective treatment is not available or simply too expensive. Also, many diseases are not sufficiently explored yet. Therefore, despite all progress, the fight against infectious diseases still remains a large and global challenge for human and veterinary medicine, even in industrialized countries with their well-developed health care systems.

This situation constantly presents politics and science with new challenges, since pathogens change and quickly adjust to new conditions. Zoonoses outbreaks are not predictable. Correlations must be recognized quickly and effective and efficient solutions and protective measures must be established.

Therefore, it is imperative that the best scientists work together interdisciplinary, both in basic research and in patient-oriented clinical and veterinary research to combine the knowledge and experience from all areas. Ebola impressively demonstrates, how quickly we must react to an outbreaks and the emergence of new pathogens. Pathogens must be identified, transmission routes and infection potential clarified, often under great timely pressure. Networked interdisciplinary work is the only way to develop effective protective measures and to stop uncontrolled proliferation.

For this research, we must establish the best possible conditions and structures. We have already accomplished a part thereof. In 2006, the German federal government adopted a research agreement on zoonoses.

Welcome Note of the Federal Ministries

This agreement forms the basis for joint research activities to ensure a successful and coordinated implementation. It was initiated when the bird flu, the influenza virus H5N1, was first detected in wild birds on the Island of Rügen in February 2006. Since then, we have witnessed outbreaks of zoonotic infectious diseases on a regular basis. In 2012, the pathogen of the year was the MERS-Corona-Virus, this year it is Ebola.

With the "National Research Platform for Zoonoses", the federal government has created a successful and future-oriented research integration tool. Because appropriate structures create significant synergies. The zoonoses research platform has proven to be a stable basis for scientific exchange.

Zoonoses are catastrophic for those afflicted, however, they also have significant effects on animal husbandry. The protection of livestock and the legitimate demand of consumers for safe food make it necessary to develop new protection strategies here as well.

This sets the bar for all inter-departmental efforts. Research results must be practicable. This shall be our joint task even in future research activities. Because "One Health Strategy" is more than a motto. It is about us, our health and the health of livestock. Zoonoses make it especially clear.

Even more reason for German politics, research and science to position themselves as an international, interdisciplinary network than before. This conference, which we highly regard, strengthens the national and international cooperation. We are pleased that this year's national Zoonoses Symposium coincides with the 7th International Conference on Emerging Zoonoses.

We wish the symposium, which was co-organized by the National Research Platform, an interesting and successful outcome. May your discussions result in new impulses for "One Health".

Dr. Joachim Klein
Federal Ministry of Education and Research

Dr. Kirsten Reinhard
Federal Ministry of Health

Dr. Ralf Rotheneder
Federal Ministry of Food and Agriculture

Committees

Scientific Committee:

Co-Chairs
Heinz Feldmann, USA
Martin Groschup, Germany
Stephan Ludwig, Germany
Jürgen A. Richt, USA
Sebastian Semler, Germany

Matthias Niedrig, Germany
Klaus Osterrieder, Germany
Lothar Wieler, Germany

Organizing Committee:

German Research Platform for Zoonoses, Germany
Target Conferences Ltd., Israel

Keynote Speakers:

Petra Dersch, Germany
Jörg Hacker, Germany
Marion Koopmanns, The Netherlands
Nikos Vasilakis, USA

General Information

Venue
Best Western Plus Hotel Steglitz International
Albrechtstraße 2, 12165 Berlin
www.si-hotel.com

Registration Information
A registration desk will operate in the foyer outside the lecture hall during the following hours:

Wednesday, October 15	18:00 – 20:00
Thursday, October 16	08:00 – 19:30
Friday, October 17	08:30 – 16:00

Name Badge
Your name badge is included in the material which you received upon registration. Please wear your badge during all Conference sessions.

Continuous Medical Education
The Joint Zoonoses 2014 Conference is registered for 12 CME points of category A by the Berlin Chamber of Physicians. Certificates will be available at the registration desk during the afternoon coffee break on Thursday and after lunch on Friday.

Continuous Veterinary Education
The Joint Zoonoses 2014 Conference is registered for 11 hours (ATF-Stunden) by the Federal Chamber of Veterinarians. Certificates will be available at the registration desk during the afternoon coffee break on Thursday and after lunch on Friday.

Oral Presentations
Oral presentations should be handed over on a common data carrier at the registration desk on Wednesday, October 15, between 09:00 and 13:00. All session rooms will be equipped with a PC computer and LCD projector. Apple computers are not available. Please make sure that you use either a powerpoint or a pdf file format.

WLAN
For internet acess pleaase register at the hotel reception on the ground floor of the hotel. WLAN will be provided without charge.

Young Scientists Breakfast
The Young Scientists Breakfast will take place at the "Pavillon"room of the hotel on Friday, Otober 17, at 08:00.

General Information

Posters
There will be two poster sessions during the conference. Poster presenters should refer to the program to find the poster session and board number assigned to them. Please use the poster board with the designated number.

***Poster Session 1*:** Thursday, October 16, 2014, 13:00 - 14:00
Presenters that are allocated to this session should hang up their posters from the morning of Thursday, October 16 and remove them by the end of the day.

***Poster Session 2*:** Friday, October 17, 2014, 13:30 - 14:30
Presenters that are allocated to this session should hang up their posters from the morning of Friday, October 17 and remove them by the end of the day.
The organizers will not be held responsible for those posters that are not removed.

Welcome Reception/Social Dinner: Thursday, October 16, 19:30
A welcome reception/social dinner will take place at the Best Western Plus Hotel. This will include dinner and drinks. All registered participants are invited to attend.

Lunch Set-up
Due to the capacity of the venue premises, lunch will be served in two consecutive shifts. Please exercise some patience while seating yourself accordingly.

Funding
The Joint Conference: German Symposium on Zoonoses Research 2014 and 7th International Conference on Emerging Zoonoses is funded by the Federal Ministry of Education and Research.
We would also like to acknowledge the following companies that have contributed towards the conference:

Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD)

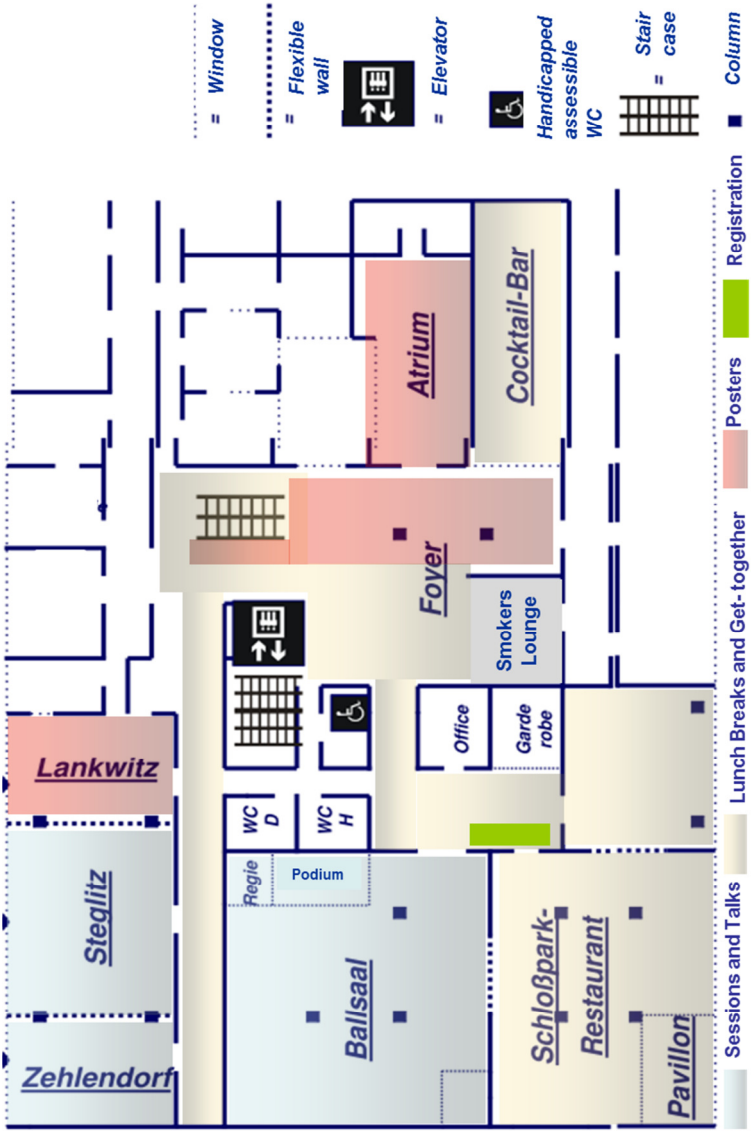
Hemispherx Biopharma, Inc.

Boehringer Ingelheim VetmedicaGmbH

Institute for Infectious Animal Diseases (IIAD)

QIAGEN Leipzig GmbH

Floorplan


= Window
= Flexible wall
= Elevator
Handicapped assessible WC
= Stair case
Column
Sessions and Talks
Lunch Breaks and Get-together
Posters
Registration
Lankwitz
Steglitz
Zehlendorf
WC D
WC H
Regie
Podium
Ballsaal
Foyer
Atrium
Cocktail-Bar
Smokers Lounge
Office
Garde robe
Schloßpark-Restaurant
Pavillon

Site Plan


CHARLOTTEN-
BURG
Straße des 17. Juni
Brandenburger Tor
Unter den Linden
Zoo
TIERGARTEN
Leipziger Str.
A 111
Messe- und Ausstellungs-gelände
Kantstr.
Kantstr.
Kurfürstendamm
A 115
WILMERS-DORF
SCHÖNEBERG
Hauptstr.
Hohenzollern-
A 100
A 104
FRIEDENAU
Flughafen Tempelhof
Tempelhofer Damm
Grunewald
SCHMARGEN-DORF
A 103
Schloßstr.
STEGLITZ
Rathaus Steglitz
Botanischer Garten
Best Western Hotel Steglitz International
Unter den Eichen
Albrechtstr.
Hamburg
Neuruppin
Stettin
Elbe
Oder
Salzwedel
A 24
A 11
A 10
A 111
A 10
Stendal
Berlin
Wolfsburg
Brandenburg
Potsdam
Fürsten-walde
A 10
A 12
A 2
A 9
Luckenwalde
A 13
Hannover
Magdeburg
Leipzig
Dresden

Program, Thursday, October 16, 2014

08:00 **Registration Opens (Poster Mounting)**

10:00 – 12:00 **PLENARY SESSION 1** *Ballroom*

Chairs: **Jürgen A. Richt**, USA
Stephan Ludwig, Germany

10:00 Opening Remarks

10:30 SYLVATIC DENGUE - THE FORGOTTEN RE-EMERGING ZOONOSIS
N. Vasilakis, USA

11:15 MERS CoV, STUDIES AT THE HUMAN ANIMAL INTERFACE
M. Koopmans, The Netherlands

12:00 *Lunch*

13:00 *Poster Viewing Session*

14:00 – 15:30 SESSION 1: *Ballroom*
(NEW AND RE-) EMERGING ZOONOTIC DISEASES PART A

Chairs: **Heinz Feldmann,** USA
Sandra Diederich, Germany

14:00 EXPERIMENTAL INFECTION OF CALVES WITH *ESCHERICHIA COLI* O104:H4
K. Hamm, S. Barth, S. Stalb, E. Liebler-Tenorio, J.P. Teifke, E. Lange, G. Kotterba, E. Dean-Nystrom, M. Bielaszewska, H. Karch, C. Menge, Germany

14:15 *IN VITRO AND IN VIVO* CHARACTERIZATION OF BOKELOH BAT LYSSAVIRUS – A RECENTLY DISCOVERED NOVEL VIRUS THAT CAUSES BAT RABIES
C. Freuling, T. Nolden, A. Banyard, D. Horton, T. Fooks, J. Teifke, T. Mettenleiter, T. Müller, Germany

14:30 THE DETECTION AND CHARACTERIZATION OF TICK-BORNE ENCEPHALITIS VIRUS STRAINS OF EUROPEAN SUBTYPE ISOLATED IN WESTERN AND EASTERN SIBERIA OF RUSSIA
I. Kozlova, **S. Tkachev**, Y. Dzhioev, M. Verkhozina, E. Doroschenko, O. Lisak, T. Demina, O. Suntsova, A. Paramonov, A. Lyapunov, A. Borisenko, N. Tikunova, D. Růžek**,** Russia

14:45 CHARACTERIZATION OF UNCULTIVABLE BAT INFLUENZA VIRUS USING A REPLICATIVE SYNTHETIC VIRUS
W. Ma, B. Zhou, J. Ma, Q. Liu, B. Bawa, W. Wang, R. Shabman, M. Duff, J. Lee, Y. Lang, J. Richt, D. Wentworth, USA

15:00 TWO INDEPENDENT EVOLUTIONARY PATHWAYS OF HPAIV
O. Stech, J. Veits, S. Abdelwhab, U. Wessels, T.C. Mettenleiter, **J. Stech**, Germany

15:15 ESTABLISHMENT OF THE INFECTION MODEL "BANK VOLE"
S. Röhrs, R.G. Ulrich, C. Drosten, J.F. Drexler, M. Keller, B. Hoffmann, D. Hoffmann, M. Beer, Germany

14:00 – 15:30 SESSION 2: ECOLOGY OF EMERGING ZOONOSES

Room Steglitz

Chairs: **Lothar H. Wieler,** Germany
Sandra Junglen, Germany

14:00 RICKETTSIA DIVERSITY IN SMALL MAMMALS FROM SOUTH AFRICA
S.S. Essbauer, M. Hofmann, D. Huhle, K. Klett, S. Mathee, Germany

14:15 GERMAN *AEDES JAPONICUS JAPONICUS (DIPTERA: CULICIDAE)* LACK VECTOR COMPETENCE FOR WEST NILE VIRUS DUE TO GENETIC DIFFERENTIATION
K. Huber, **S. Jansen**, K. Schuldt, M. Rudolf, M. Leggewie, M. Badusche, D.M. Fonseca, A. Krüger, E. Tannich, S.C. Becker, Germany

14:30 EFFECTS OF LANDSCAPE AND MOSQUITO COMMUNITY ON WEST NILE VIRUS INCIDENCE IN WILD BIRDS
M. Ferraguti, J. Martínez-de la Puente, D. Roiz, S. Ruiz, R. Soriguer, J. Figuerola, Spain

14:45 FERAK VIRUS AND JONCHET VIRUS REPRESENT TWO NOVEL LINEAGES OF INSECT-RESTRICTED BUNYAVIRUSES
M. Marklewitz, F. Zirkel, A. Kurth, C. Drosten, S. Junglen, Germany

15:00 GLOBAL CHANGES AND WILDLIFE ZOONOTIC DISEASES EMERGENCE: THE CASE OF TICK-BORNE ENCEPHALITIS (TBE)
A. Rizzoli, D. Arnoldi, L. Bolzoni, F. Cagnacci, H.C. Hauffe, M. Neteler, C. Rossi, V. Tagliapietra, R. Rosà, Italy

15:15 NOVEL DIVERGENT VIRUSES DISCOVERED IN MOSQUITOES CAPTURED FROM DIFFERENT COUNTRIES OF EUROPE AND SENEGAL
A. Vázquez, M. Wiley, L. Cuevas, L. Herrero, S. Ruiz, M. Diallo, M. Grisenti, E. Pérez-Pastrana, L. Orshan, G. Fall, R.C. Soriguer, A. Rizzoli, H. Bin, N. Nowotny, G. Palacios, A. Tenorio, M.P. Sánchez-Seco, Spain

14:00 – 15:30 SESSION 3: EPIDEMIOLOGY, MODELING AND PREDICTION

Room Zehlendorf

Chairs: **Lothar Kreienbrock,** Germany
Sophie Rettenbacher-Riefler, Germany

14:00 RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY AGAINST *TOXOPLASMA GONDII*: RESULTS FROM THE FIRST REPRESENTATIVE SEROSURVEY OF ADULTS IN GERMANY
H. Wilking, M. Thamm, A. Aebischer, K. Stark, F. Seeber, Germany

14:15 SUCCESS IN DIVERSITY - INSIGHTS INTO THE POPULATION STRUCTURE OF *E. COLI*
T. Semmler, T.R. Connor, D.J. Pickard, R.A. Kingsley, N. Ahmed, C. Ewers, G. Dougan, N.R. Thomson, J. Corrander, L.H. Wieler, Germany

14:30 EARLY WARNING OF WEST NILE VIRUS MOSQUITO VECTOR: CLIMATE AND LAND USE MODELS SUCCESSFULLY EXPLAIN PHENOLOGY AND ABUNDANCE OF *CULEX PIPIENS* MOSQUITOES IN NORTH-WESTERN ITALY
R. Rosà, G. Marini, L. Bolzoni, M. Neteler, M. Metz, L. Delucchi, E.A. Chadwick, L. Balbo, A. Mosca, M. Giacobini, L. Bertolotti, A. Rizzoli, Italy

14:45 DISENTANGLING THE ECOLOGICAL CONDITIONS AFFECTING WEST NILE VIRUS HAZARD IN THE OLD WORLD
M. Marcantonio, A. Rizzoli, M. Metz, R. Rosà, G. Marini, E. Chadwick, M. Neteler, Italy

15:00 AN EPIDEMIOLOGICAL STUDY OF CANINE ECHINOCOCCOSIS AND LIVESTOCK HYDATIDOSIS IN SUDAN
N. Abass, M. Herzig, G. Boubaker, A.A. Elfadil, F.J. Conraths, Germany

14:00 – 15:30 **SESSION 3: EPIDEMIOLOGY, MODELING AND PREDICTION *Continued*** *Room Zehlendorf*

15:15 THE POTENTIAL FOR RESPIRATORY DROPLET–TRANSMISSIBLE A/H5N1 INFLUENZA VIRUS TO EVOLVE IN A MAMMALIAN HOST
J.M. Fonville, C. Russell, A. Brown, D. Burke, D. Smith, S. James, S. Herfst, S. van Boheemen, M. Linster, E. Schrauwen, L. Katzelnick, A. Mosterin, T. Kuiken, E. Maher, G. Neumann, A. Osterhaus, Y. Kawaoka, R. Fouchier, D. Smith, UK

15:30 *Coffee Break and Poster Viewing*

16:00 AWARD OF THE GERMAN ACADEMY OF VETERINARY HEALTH (AKADEMIE FUER TIERGESUNDHEIT)

16:30 – 17:30 **CURRENT EBOLA VIRUS OUTBREAK IN WEST AFRICA** *Ballroom*

17:30 – 19:30 **General Assembly: German Research Platform for Zoonoses (IN GERMAN)** *Room Steglitz-Zehlendorf*

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

08:00 – 09:00 YOUNG SCIENTISTS BREAKFAST *Pavillon Room*

09:00 – 10:30 SESSION 4: (NEW AND RE-) EMERGING ZOONOTIC DISEASES PART B *Ballroom*

Chairs: **Marcel A. Müller,** Germany
Linda Brunotte, Germany

09:00 CLINICAL COURSE OF INFECTION AND TISSUE TROPISM OF HEPATITIS C VIRUS-LIKE HEPACIVIRUSES IN HORSES
S. Walter, S. Pfaender, J.M.V. Cavalleri, J. Doerrbecker, B. Campana, R.J.P. Brown, P.D. Burbelo, A. Postel, K. Hahn, A. Kusuma, N. Riebesehl, W. Baumgärtner, P. Becher, M.H. Heim, T. Pietschmann, K. Feige, E. Steinmann, Germany

09:15 SALMONELLA CO-OPTS FOR CMA FOR ITS INTRACELLULAR GROWTH AND SURVIVAL
V. Singh, J. Finke, P. Schwerk, K. Tedin, Germany

09:30 THE TICK-BORNE PATHOGEN *CANDIDATUS NEOEHRLICHA MIKURENSIS* IN *IXODES RICINUS* AND NATURAL VERTEBRATE HOSTS IN SOUTHERN SWEDEN
M. Andersson, Sweden

09:45 *CULEX PIPIENS* AND *CULEX TORRENTIUM* MOSQUITOES FROM GERMANY HAVE VECTOR COMPETENCE FOR WEST NILE VIRUS
M. Leggewie, M. Badusche, M. Rudolf, S. Jansen, J. Börstler, K. Huber, E. Tannich, A. Krüger, J. Schmidt-Chanasit, S.C. Becker, Germany

10:00 VIRUS DISCOVERY IN MOSQUITOES FROM THE NEOTROPICS
A. Kopp, A. Hübner, F. Zirkel, D. Hobelsberger, A. Estrada, T. Gillespie, C. Drosten, S. Junglen, Germany

10:15 DETECTION OF NGARIVIRUS, A HIGHLY VIRULENT ORTHOBUNYAVIRUS IN SMALL RUMINANTS FROM MAURITANIA
M. Eiden, A. Vina-Rodriguez, B.O. El Mamy, K. Isselmou, U. Ziegler, D. Höper, S. Jäckel, A. Balkema-Buschmann, H. Unger, B. Doumbia, M.H. Groschup, Germany

09:00 – 10:30 SESSION 5: NOVEL METHODS AND DIAGNOSTICS

Room Steglitz

Chairs: **Jürgen A. Richt**, USA
Anne Balkema-Buschmann, Germany

09:00 ANTI-IDIOTYPIC ANTIBODIES IN DIAGNOSIS OF OPISTHORCHIASIS
A. Bulashev, S. Serikova, S. Eskendirova, Kazakhstan

09:15 DEVELOPMENT OF A CELL CULTURE SYSTEM FOR HEPATITIS E VIRUS
R. Johne, E. Trojnar, J. Reetz, P. Nickel, R.G. Ulrich, P. Machnowska, J. Sachsenroeder, J. Hofmann, Germany

09:30 EVALUATION OF AN INDIRECT ELISA USING A TACHYZOITE SURFACE ANTIGEN SAG1 FOR DIAGNOSIS OF *TOXOPLASMA GONDII* INFECTION IN CATS
F. Hosseini, M. Hosseininejad, Iran

09:45 *MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS* SHEEP STRAIN JIII-386: SEQUENCING, ASSEMBLING, ANNOTATION, AND GENOME COMPARISON
P. Möbius, M. Hölzer, M. Felder, G. Nordsiek, M. Groth, H. Köhler, K. Reichwald, M. Platzer, M. Marz, Germany

10:00 HYGIENISATION OF *MYCOBACTERIUM FORTUITUM* IN CATTLE MANURE THROUGH LACTIC ACID FERMENTATION
H.A. Scheinemann, M. Krüger, Germany

10:15 ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR DETECTION OF ANTIBODIES TO THE NOVEL LLOVIU (FILOVIRUS) AND CRIMEAN CONGO HEMORRHAGIC FEVER VIRUSES LOCATED IN SPAIN
E. Ramírez de Arellano, M. Ortiz, F. Lasala, P. López, M.J. Perteguer, L. Fernández, M.Á. Habela, A. Estrada-Peña, G. Palacios, M.P. Sánchez-Seco, A. Tenorio, A. Negredo, Spain

09:00 – 10:30 SESSION 6: PATHOGEN-CELL INTERACTION AND IMMUNITY

Room Zehlendorf

Chairs: **Allison Groseth,** USA
Wenjun Ma, USA

09:00 INEFFICIENT CELLULAR TRANSPORT OF THE ATTACHMENT GLYCOPROTEIN OF THE AFRICAN HENIPAVIRUS M74 PLAYS A MAJOR ROLE FOR THE RESTRICTED FUNCTIONAL ACTIVITY
N. Krüger, M. Hoffmann, M.A. Müller, J.F. Drexler, C. Drosten, G. Herrler, Germany

09:15 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) TREATMENT AMELIORATES *TOXOPLASMA GONDII*- INDUCED ENCEPHALITIS IN MICE
A. Parlog, L. Möhle, A. Biswas, D. Reglodi, **M.M. Heimesaat**, I.R. Dunay, Germany

09:30 FLU´S FIRST DEFENSE AGAINST IFN: VIRAL SUPPRESSORS OF TYPE I IFN RESPONSE ARE PREPACKAGED IN INFLUENZA A VIRUS VIRIONS
S. Liedmann, E.R. Hrincius, C. Guy, D. Anhlan, R. Dierkes, G. Wu, D.R. Green, T. Wolff, J.A. McCullers, S. Ludwig, C. Ehrhardt, Germany

09:45 MOLECULAR BASIS FOR DISRUPTION OF E-CADHERIN ADHESION BY BOTULINUM NEUROTOXIN A COMPLEX
K. Lee, X. Zhong, S. Gu, A.M. Kruel, M.B. Dorner, K. Perry, **A. Rummel**, M. Dong, R. Jin, Germany

10:00 NOVEL IMMUNOSTIMULATORY FLAGELLIN-LIKE PROTEIN FLAC IN *CAMPYLOBACTER JEJUNI* AND OTHER *CAMPYLOBACTERALES*
E. Leno, E. Gripp, S. Maurischat, B. Kaspers, K. Tedin, S. Klose, **C. Josenhans**, Germany

09:00 – 10:30 SESSION 6: PATHOGEN-CELL INTERACTION AND IMMUNITY *Continued* *Room Zehlendorf*

10:15 HUMAN CORONAVIRUS REPLICATION IS CYCLOPHILIN A-DEPENDENT AND INHIBITED BY NOVEL NON-IMMUNOSUPPRESSIVE CYCLOSPORINE A-DERIVATIVES INCLUDING ALISPORIVIR AND NIM811
A. Von Brunn, J. Carbajo-Lozoya, Y. Ma-Lauer, B. von Brunn, P. Mayrhofer, R. Hilgenfeld, S. Kallies, D. Muth, M.A. Müller, C. Drosten, S. Ciesek, M. Malešević, G. Fischer, Germany

10:30 Coffee Break and Poster Viewing

11:00 – 12:30 SESSION 7: RESERVOIRS OF ZOONOSES *Room Steglitz*

Chairs: **Sandra S. Essbauer,** Germany
Claudia Kohl, Germany

11:00 SEROLOGICAL EVIDENCE OF INFLUENZA A VIRUSES IN BATS FROM AFRICA
G.S. Freidl, T. Binger, M.A. Müller, E. de Bruin, J. van Beek, V.M. Corman, A. Rasche, J.F. Drexler, A. Annan, S. Oppong, Y. Adu-Sarkodie, M. Tschapka, V.M. Cottontail, M. Knörnschild, C. Drosten, M. Koopmans, The Netherlands, Germany and Ghana

11:15 EPITHELIAL CELL LINES FROM BATS, RODENTS AND INSECTIVORES – A NOVEL TOOL FOR *IN VITRO* INVESTIGATION OF PATHOGEN-HOST INTERACTION
I. Eckerle, R. Ulrich, A. Rang, B. Klempa, L. Radosa, M.A. Müller, C. Drosten, Germany

11:30 EXPLORING THE METABOLIC INTERFACE BETWEEN THE GASTROINTESTINAL PATHOGEN CAMPYLOBACTER, ITS HOST AND THE MICROBIOTA
J. Mohr, H. Vorwerk, C. Huber, P. Grüning, K. Methling, A. von Altrock, O. Wensel, S. Bhuju, J. Kamphues, T. Alter, A. Flieger, K. Schmidt-Hohagen, D. Schomburg, M. Lalk, W. Eisenreich, P. Valentin-Weigand, **D. Hofreuter**, Germany

11:00 – 12:30 SESSION 7: RESERVOIRS OF ZOONOSES *Continued* *Room Steglitz*

11:45 CLONALITY AMONG ESBL-PRODUCING *E. COLI* OF SEQUENCE TYPES (ST) 131, ST410 AND ST167 ISOLATED FROM HUMAN CLINICAL AND AVIAN WILDLIFE SAMPLES FROM THE CITY OF BERLIN, GERMANY
M. Wöhrmann, K. Schaufler, K. Müller, T. Semmler, R. Leistner, A. Kola, L.H. Wieler, **S. Guenther**, Germany

12:00 VARIOUS WAYS OF SHEDDING OF BORNA DISEASE VIRUS IN LIVING BICOLORED WHITE-TOOTHED SHREWS
D. Nobach, M. Bourg, S. Herzog, H. Lange-Herbst, J.A. Encarnação, M. Eickmann, C. Herden, Germany

12:15 SURVEILLANCE OF ERADICATION OF MRSA AND ESBL-E ON A MODEL PIG FARM
R. Schmithausen, S. Kellner, S. Schulze-Geisthoevel, S. Hack, G. Bierbaum, A. Hoerauf, M. Exner, B. Petersen, I. Bekeredjian-Ding, Germany

11:00 – 12:30 SESSION 8: RISK ASSESSMENT & PUBLIC HEALTH

Ballroom

Chairs: **Robin Koeck, Germany**
Szilvia Vincze, Germany

11:00 MOLLUSCAN VIBRIO SPECIES IN CANADA'S ESTUARIES: A. LONGITUDINAL STUDY OF TREND AND DYNAMICS IN TEMPERATE WATERS
S.K. Banerjee, J.M. Farber, Canada

11:15 AN OBSERVATIONAL STUDY OF AN OUTBREAK OF MERS-CORONAVIRUS IN JEDDAH, KSA, IN 2014
D. Muth, V. Corman, R. Hussain, H. Madani, A. Zumla, A. Al Shangiti, Z. Memish, C. Drosten, Germany

11:30 TRICHINELLOSIS – A NEGLECTED ZOONOSIS IN GERMANY?
K. Nöckler, S. Reckinger, A. Mayer-Scholl, Germany

11:45 EFFICACY TESTING OF INACTIVATION METHODS FOR FILOVIRUSES
E. Haddock, F. Feldmann, H. Feldmann, USA

12:00 ESBL-PRODUCING *E. COLI* FROM ANIMAL AND HUMAN SOURCES – WHAT DO THEY SHARE?
H. Sharp, L. Valentin, K. Hille, U. Seibt, J. Fischer, Y. Pfeifer, G. Brenner Michael, S. Nickel, J. Schmiedel, L. Falgenhauer, A. Friese, R. Bauerfeind, U. Rösler, C. Imirzalioglu, T. Chakraborty, R. Helmuth, G. Valenza, G. Werner, S. Schwarz, B. Guerra-Roman, B. Appel, L. Kreienbrock, A. Käsbohrer, Germany

12:15 MRSA INFECTIONS IN COMPANION ANIMALS: CHARACTERIZATION OF RISK FACTORS
S. Vincze, A.G. Brandenburg, W. Espelage, I. Stamm, L.H. Wieler, P.A. Kopp, A. Lübke-Becker, B. Walther, Germany

11:00 – 12:30 SESSION 9: VARIOUS TOPICS *Room Zehlendorf*

Chairs: **Klaus Osterrieder**, Germany
Marketa Derdáková, Slovakia

11:00 PATHOGENIC NEMATODES FOUND IN FISH AND FISHERY PRODUCTS MADE OF PINK SALMON (ONCORHYNCHUS GORBUSCHA) FROM MARKETS IN POLAND
M. Rozycki, E. Bilska-Zajac, E. Chmurzynska, T. Cencek, M. Kochanowski, Poland

11:15 MOLECULAR DETERMINANTS OF VIRULENCE OF EMERGING TICK-BORNE PHLEBOVIRUSES: FROM PHLYLOGENETIC TREES TO THE MOLECULAR BASIS OF PATHOGENESIS
K. Matsuno, C. Matysiak, C. Weisend, **A. Groseth**, H. Ebihara, USA

11:30 AN INFECTIOUS BAT CHIMERIC INFLUENZA VIRUS HARBORING THE ENTRY MACHINERY OF A CONVENTIONAL INFLUENZA A VIRUS
M. Juozapaitis, É. Aguiar Moreira, I. Mena, S. Giese, D. Riegger, A. Pohlmann, D. Höper, G. Zimmer, M. Beer, A. García-Sastre, **M. Schwemmle**, Germany

11:45 CHARACTERIZATION OF GUAROA VIRUS GENETIC DIVERSITY, EVOLUTION AND SPREAD
A. Groseth, K.K. Wollenberg, V. Mampilli, T. Shupert, C. Matysiak, C. Weisend, T.J. Kochel, R.B. Tesh, H. Ebihara, USA

12:00 EFFECTS OF CATHELICIDIN ANTIMICROBIAL PEPTIDES AGAINST LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*
S. Blodkamp, K. Kadlec, H.Y. Naim, S. Schwarz, **M. von Köckritz-Blickwede**, Germany

12:30 *Lunch*

13:30 *Poster Viewing*

14:30 – 16:00 PLENARY SESSION 2 *Ballroom*

Chair: **Martin Groschup**, Germany

14:30 CHALLENGES FOR ZOONOSES RESEARCH IN GERMANY AND WORLDWIDE
J. Hacker, Germany

15:15 NEW HOST SPECIFIC INFECTION BIOLOGY ASPECTS OF YERSINIA ENTEROCOLITICA
P. Dersch, Germany

16:00 – 16:30 POSTER AWARDS AND CLOSING CEREMONY *Ballroom*

ORAL PRESENTATIONS

SYLVATIC DENGUE - THE FORGOTTEN RE-EMERGING ZOONOSIS

N. Vasilakis

Department of Pathology, University of Texas Medical Branch, Galveston, United States

The four dengue virus (DENV) serotypes that circulate among humans emerged independently from ancestral sylvatic progenitors present in non-human primates (NHPs), following the establishment of human populations large and dense enough to support continuous human transmission by Aedes spp mosquitoes. This ancestral sylvatic cycle is still extant, maintained in NHPs and arboreal Aedes mosquitoes in the forests of Southeast Asia and West Africa. Todate the spread of sylvatic DENV to humans was limited and sporadic. However, counteracting the rise of herd immunity, there are the extensive and ongoing land use changes throughout SE Asia, which have resulted in a shift in agriculture and concomitant human traffic into the forest, which will likely result in increased human contact with the sylvatic cycle and with it re-emergence potential. Herein an overview of the ecology, molecular evolution, and potential for adaptation of sylvatic DENV to human transmission is provided.

EXPERIMENTAL INFECTION OF CALVES WITH ESCHERICHIA COLI O104:H4

K. Hamm[1], S. Barth[1], S. Stalb[1], E. Liebler-Tenorio[1], J.P. Teifke[2], E. Lange[2], G. Kotterba[3], E. Dean-Nystrom[4], M. Bielaszewska[5], H. Karch[5], C. Menge[1]

[1]Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany, [2]Friedrich-Loeffler-Institut, Department of Experimental Animal Facilities and Biorisk Management, Greifswald/ Isle of Riems, Germany, [3]Friedrich-Loeffler-Institut, Institute of Infectology, Greifswald/ Isle of Riems, Germany, [4]U. S. Department of Agriculture, Food Safety and Enteric Pathogens Research Unit, N a D C, Ames, United States, [5]University of Münster, Institute for Hygiene, Münster, Germany

A major human outbreak of hemolytic-uremic syndrome in Germany 2011 could be traced back to an unusual EHEC strain of serotype O104:H4 never detected in cattle, the primary STEC/EHEC reservoir, before. To assess if EHEC O104:H4 can utilize ruminants as reservoir, we determined the clinical appearance, the pattern and magnitude of fecal shedding and the site of colonization in a bovine infection model. Fifteen (five per strain) 100-day-old weaned calves were inoculated with 10^10 CFU of EHEC O104:H4, of EHEC O157:H7 (positive control) or of non-pathogenic *E. coli* O43:H28 (negative control) and necropsied 4 dpi. EHEC O157 and O104 were recovered in equal numbers (approx. 5 x 10^5 CFU/g feces) from feces 4 dpi whereas the non-pathogenic *E. coli* was detected in much lower numbers (approx. 5 x 10^2 CFU/g feces). EHEC O104 was recovered from intestinal content as well as from mucosal tissue samples even though histologic examinations of multiple intestinal sites failed to detect O104 bacteria in proximity of intestinal epithelial cells. In a second trial, 15 calves were inoculated the same way and necropsied 28 dpi. EHEC O157:H7 was recovered from the feces, intestinal content and associated with intestinal tissue even at day 28 pi. By contrast, EHEC O104:H4 was recovered in quantifiable numbers from fecal samples until day 24 only. Analyses of sequential fecal samples demonstrated that increasing percentages of colonies of inoculum-type bacteria (O104:H4) were formed by bacteria that had lost the pAA plasmid. These results are the first evidence that cattle can carry EHEC O104:H4 at least transiently, although EHEC O104:H4 appears to be less well adapted to bovines as classical STEC strains.

IN VITRO AND *IN VIVO* CHARACTERIZATION OF BOKELOH BAT LYSSAVIRUS – A RECENTLY DISCOVERED NOVEL VIRUS THAT CAUSES BAT RABIES

C. Freuling[1], T. Nolden[1], A. Banyard[2], D. Horton[2], T. Fooks[2], J. Teifke[3], T. Mettenleiter[1], T. Müller[1]

[1]Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany, [2]Wildlife Zoonoses and Vector Borne Diseases Research Group, Animal Health and Veterinary Laboratories Agency, Weybridge, United Kingdom, [3]Department of Animal Husbandry and Biorisk Management, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany

Bat rabies in Europe poses a low but undeniable risk to human health. Besides the European Bat Lyssaviruses 1 and 2 (EBLV-1 and 2) responsible for the vast majority of bat rabies cases reported in Europe, Bokeloh bat lyssavirus (BBLV), was recently isolated from Natterer's bats (Myotis nattererii), a chiropteran species with a widespread and abundant distribution across Europe. As a novel lyssavirus, the risks of BBLV to animal and human health are unknown and as such characterization both *in vitro* and *in vivo* was required to assess pathogenicity and vaccine protection. Full genome sequence analysis and antigenic cartography demonstrated that the German BBLV isolates are most closely related to EBLV-2 and Khujand virus and can be placed within phylogroup I. *In vivo* characterization demonstrated that BBLV was pathogenic in mice when inoculated peripherally causing clinical signs typical for rabies encephalitis, with higher pathogenicity observed in juvenile mice. Similar to other lyssaviruses, BBLV infection caused a mild lympho-plasmocytic encephalitis. Interestingly, in comparison with EBLV-1, the accumulation of viral antigen in the neuronal cytoplasm was less pronounced in BBLV infected animals. There were no significant differences in onset of disease, mean survival time, body mass reduction and the development of rabies clinical signs between the two isolates used. A limited vaccination challenge experiment in mice was also conducted and suggested that current vaccines would afford some protection against BBLV although further studies are warranted to determine a serological cut off for protection.

THE DETECTION AND CHARACTERIZATION OF TICK-BORNE ENCEPHALITIS VIRUS STRAINS OF EUROPEAN SUBTYPE ISOLATED IN WESTERN AND EASTERN SIBERIA OF RUSSIA

I. Kozlova[1,2], S. Tkachev[3], Y. Dzhioev[1,4], M. Verkhozina[5], E. Doroschenko[1], O. Lisak[1], T. Demina[6], O. Suntsova[1], A. Paramonov[1], A. Lyapunov[1], A. Borisenko[1], N. Tikunova[3], D. Růžek[7,8]

[1]Laboratory of Molecular Epidemiology and Genetic Diagnostics, Fssfe Scientific Centre of Family Health and Human Reproduction Problems Sb Rams, Irkutsk, Russian Federation, [2]Department of Microbiology, Virology and Immunology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [3]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine Sb Ras, Novosibirsk, Russian Federation, [4]Laboratory of Molecular Virology and Biotechnology, Institute of Biomedical Technology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [5]Department of Virology Research and Pcr Laboratory, Centre for Epidemiology and Hygiene in Irkutsk Region, Irkutsk, Russian Federation, [6]Faculty of Veterinary Medicine and Biotechnology, Irkutsk State Academy of Agriculture, Irkutsk, Russian Federation, [7]Laboratory of Arbovirology, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, [8]Department of Virology, Veterinary Research Institute, Brno, Czech Republic

Currently, tick-borne encephalitis virus (TBEV) is divided into three genotypes (subtypes): 1) Far-Eastern 2) Western (or European); 3) Siberian [Ecker et al., 1999]. Each subtype has its own habitat area where its absolute domination is observed.

TBEV of Western subtype has the broad habitat area extending from Europe to Asia. The eastern boundary of this subtype distribution is South Korea [Kim, 2008; Yun, 2009]. The eastern known border of TBEV Western subtype in Russia is the territory of Eastern Siberia. With use of molecular hybridization of nucleic acids method with genotype-specific probes and sequencing of virus genome fragments 13 strains of Western subtype were found in Siberia. Five of them were isolated in the Western Siberia territory (Altai region), and eight in the territory of Eastern Siberia (Irkutsk region). Eight strains were isolated from Ixodes persulcatus (Schulze, 1930) ticks, four strains from small mammals of different species and one strains was isolated from blood sample from patient with feverish form of tick-borne encephalitis.

The estimation of some biological properties of isolated TBEV strains was performed. Two strains demonstrated high cerebral and peripheral activity and possessed high invasive characteristics that could demonstrate their ability to cross the blood brain barrier. Moreover the wide spectrum of plaques size in PK cell culture was demonstrated for different strains. For five strains the rct37 and rct42 genetic markers were determined. Four of five strains demonstrated high growth in PK cell cultures both at 37ºC and at 42ºC, that indicates their good adaptive abilities.

The study was supported by Russian Scientific Foundation (project №14-15-00615).

CHARACTERIZATION OF UNCULTIVABLE BAT INFLUENZA VIRUS USING A REPLICATIVE SYNTHETIC VIRUS

W. Ma[1], B. Zhou[2], J. Ma[1], Q. Liu[1], B. Bawa[1], W. Wang[2], R. Shabman[2], M. Duff[1], J. Lee[1], Y. Lang[1], J. Richt[1], D. Wentworth[2]

[1]Department of Diagnostic Medicine/pathobiology, Kansas State University, Manhattan, Ks, United States, [2]Virology, J. Craig Venter Institute, Rockville, Md, United States

Bats harbor many viruses, which are periodically transmitted to humans resulting in outbreaks of disease (e.g., Ebola, SARS-CoV). Recently, influenza virus-like sequences were identified in bats; however, the viruses could not be cultured. This discovery aroused great interest in understanding the evolutionary history, and pandemic potential of bat-influenza virus. Using synthetic genomics, we were unable to rescue the wild type bat virus, but could rescue a modified bat influenza virus that had the HA and NA coding regions replaced with those of A/PR/8/1934. This modified bat-influenza virus replicated efficiently *in vitro* and in mice, resulting in severe disease. Unlike other influenza viruses, engineering truncations hypothesized to reduce interferon antagonism into the NS1 protein didn't attenuate bat-influenza. In contrast, substitution of a putative virulence mutation from the bat-influenza PB2 significantly attenuated the virus in mice and introduction of a putative virulence mutation increased its virulence. Minigenome replication studies and virus reassortment experiments demonstrated that bat-influenza has very limited genetic and protein compatibility with Type A or Type B influenza viruses, yet it readily reassorts with another divergent bat-influenza virus. Collectively, our data indicate that the bat-influenza virus is a real virus that poses little, if any, pandemic threat to humans; and also provide new insights into the evolution and basic biology of influenza viruses.

TWO INDEPENDENT EVOLUTIONARY PATHWAYS OF HPAIV

O. Stech, J. Veits, S. Abdelwhab, U. Wessels, T.C. Mettenleiter, J. Stech

Institute for Molecular Virology and Cellular Biology, Friedrich-Loeffler-Institut (FLI), Greifswald Isle of Riems, Germany

HPAIV develop from low-pathogenic precursors by acquisition of a polybasic HA cleavage site (HACS). Beside this prime virulence determinant, additional adaptive changes might accumulate in those precursors prior to emergence of an HPAIV. Here, we aimed to unravel the genetic determinants which, beside the polybasic HACS, enable transformation of LPAIV into HPAIV.
To select a minimal gene constellation sufficient for a high virulence, we co-transfected plasmids coding for all eight genes from an H5N1 HPAIV and seven, except HA, from an H5N1 LPAIV, and used the supernatant to infect chickens. Shed reassortants carried the HPAIV PB2, NP, HA, NA, and M genes; a reconstituted virus was highly pathogenic and transmissible like the wild-type. Furthermore, by stepwise exchange of the HPAIV genes, we eventually found that an LPAIV reassortant carrying only the HPAIV HA and NA contains the minimum set of HPAIV genes enabling high virulence. Furthermore, abolishing the NA stalk deletion led to considerably reduced lethality and no transmission in chicken. Conversely, an LPAIV reassortant carrying only the HPAIV HA but the LPAIV NA with engineered stalk deletion displayed 100% lethality both after primary or contact infection. Therefore, the NA stalk deletion is an essential virulence determinant beside the polybasic HACS. Remarkably, the LPAIV NA which contains no stalk deletion, introduced into the H5N1 HPAIV exclusively, did not reduce virulence and transmission compared to the HPAIV parent virus. Therefore, NA stalk deletion and polybasic HACS form a minimum set of virulence determinants. However, our selection experiment in chickens, infected with transfection supernatants, indicates that beside the HPAIV NA, the PB2, NP, and M enable maximal virulence.
Data mining in all public PB2, NP, and M1 protein sequences revealed that there are natural HPAIV strains corresponding to the HA/NA reassortant, confirming that polybasic HACS and NA stalk deletion form minimum set of virulence determinants. However, natural HPAI strains without stalk deletion but with several NP mutations from the HPAIV studied, corresponding to the NA/HPAIV reassortant, indicate an alternative set of virulence determinants. Therefore, those two sets of virulence determinants indicate two independent pathways of evolution of LPAIV into HPAIV.

ESTABLISHMENT OF THE INFECTION MODEL "BANK VOLE"

S. Röhrs[1], R.G. Ulrich[2], C. Drosten[3], J.F. Drexler[3], M. Keller[2], B. Hoffmann[1], D. Hoffmann[1], M. Beer[1]
[1]Friedrich-Loeffler-Institut, Institute for Diagnostic Virology, Greifswald Riems, Germany, [2]Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald Isle of Riems, Germany, [3]University of Bonn Medical Centre, Institute of Virology, Bonn, Germany

The bank vole (Myodes glareolus) is one of the most dominating rodent species within forest habitats in Europe. Bank voles are known to harbor different zoonotic pathogens that can cause disease in humans. Puumala hantavirus (PUUV), cowpox virus (CPXV) and different bacterial pathogens have been detected in bank voles. Recently, novel human hepatitis C virus (HCV)-like agents (genus Hepacivirus) were discovered in different rodent species, including bank voles.
Numerous pathogens in wild rodents have already been traced, but little is known about the various aspects of host-pathogen interaction, such as pathogenicity, persistence, immune response and transmission; aspects that could be studied under laboratory conditions when having an appropriate animal model. To this end, an animal model based on a bank vole colony will be established at the Friedrich-Loeffler-Institut in Germany.
Three different viruses will be used as initial pathogen models: PUUV frequently cause clusters and small outbreaks in humans and is a "typical zoonotic" agent causing a persistent infection in the bank vole reservoir; CPXV infections of humans are mainly caused by contact with pets such as rats or cats, but wild rodents, and voles in particular, are discussed as reservoir hosts; the newly detected bank vole-associated hepacivirus is e.g. a promising candidate for developing an animal model for experimental research on antiviral interventions against HCV infections in humans.
The new infection model "bank vole" will be an important tool for zoonosis research - not only for viruses but also for other infectious pathogens like bacteria or parasites.

RICKETTSIA DIVERSITY IN SMALL MAMMALS FROM SOUTH AFRICA

S.S. Essbauer[1], M. Hofmann[1], D. Huhle[1], K. Klett[1], S. Mathee[2]

[1]Department of Virology and Rickettsiology, Bundeswehr Institute of Microbiology, Munich, Germany, [2]Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

Rickettsioses are recognized as emerging infections in several parts of the world. Known Rickettsia species that are transmitted by ticks in South Africa are *R. conorii* and *R. africae*. We recently showed that small mammals' ears are valid tissues to investigate rickettsia. In this study we collected small mammals originating from 35 localities, representing six different vegetation types, in South Africa and investigated these for the presence of rickettsial DNA. In summary, ear samples from 1616 small mammals belonging to 13 genera were collected. Nucleic acid was isolated and thereafter screening for rickettsial DNA in a pan rickettsia real-time PCR. Positive samples were investigated by multi-locus sequence typing of ompA, ompB and 23S RNA fragments in order to determine the respective Rickettsia species. From the 1616 DNAs 251 (15.5%) were positive in the initial real-time screening PCR. The prevalence of rickettsial DNA was highest at the following localities: Somerset West (n=4/8, 50%), Wolwedans (n=10/22, 45.5%), Anysberg (n=22/52, 42.3%), Rietvlei, Gauteng (n=23/51, 45.1%) and Goegap (n=16/42, 38.1%). Interestingly, rickettsial DNA could be detected in almost all investigated small mammal species and mostly in the rodent Rhabdomys sp. (n=127/251, 50.1%). At least 11 Rickettsia species were recorded, including *R. conorii, R. massiliae, R. felis* and *R. helvetica*. Further by multi-locus sequence typing six new rickettsia species were recorded. One species was only found once whereas the other five seemed to have a ubiquitous presence in South Africa. In conclusion, a surprisingly high diversity of Rickettsia species were recorded in small mammals in South Africa. The study confirms the role of small mammals as reservoir for rickettsia.

GERMAN *AEDES JAPONICUS JAPONICUS (DIPTERA: CULICIDAE)* LACK VECTOR COMPETENCE FOR WEST NILE VIRUS DUE TO GENETIC DIFFERENTIATION

K. Huber[1,2,3], S. Jansen[1], K. Schuldt[4], M. Rudolf[1], M. Leggewie[1], M. Badusche[1], D.M. Fonseca[5], A. Krüger[6], E. Tannich[7,8], S.C. Becker[1,9]

[1]Molekulare Entomologie, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]German Mosquito Control Association, (Kabs/gfs), Waldsee, Germany, [3]Centre for Organismal Studies, Universität Heidelberg, Heidelberg, Germany, [4]Molekulare Medizin, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [5]Rutgers University, Center for Vector Biology, New Brunswick, Nj, United States, [6]Med. Entomologie, Bundeswehrkrankenhaus, Hamburg, Germany, [7]Molekulare Parasitologie, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [8]Partner Site Hamburg Luebeck Borstel, German Centre for Infection Research, Hamburg, Germany, [9]Institut Für Infektionsmedizin, Friedrich-Loeffler-Institut, Greifswald Isle of Riems, Germany

The interplay between arthropod-borne (arbo) viruses and their vectors is usually complex and often exerts unique relationships. For several arboviruses transmission is restricted to very few mosquito species. Furthermore, differences in vectorial capacity between geographically isolated mosquito populations of the same species point to specific genetic traits that allow efficient pathogen transmission. *Aedes japonicus japonicus*, an invasive mosquito species in the USA and Europe has proven vector competence for West Nile virus (WNV) in the USA. Currently the species is expanding its range throughout Germany. On the other hand, WNV an emerging arbovirus originated from Africa is already circulating in several European countries and might be soon introduced into Germany. Because newly introduced and rapidly expanding vector species might pose a potential risk for public health in Germany, we assessed genetic makeup and the vectorial capacity of German *Aedes japonicus japonicus* populations for WNV and Japan Encephalitis virus (JEV). The results indicate that German *Aedes japonicus japonicus* are susceptible for JEV (100% infection rate at day 14) but are resistant to infection with WNV. Of 67 *Aedes japonicus japonicus* females tested for WNV infection on day 14, none had measurable amounts of WNV-RNA (0% infection rate). The WNV resistance is independent from co-infection with other flaviviruses or the presence of endosymbiotic *Wolbachia* since we found no evidence for other flavivirus infections nor detectable *Wolbachia* infection. Most probably the absence of vector competence of German *Aedes japonicus japonicus* is due to genetic differences of the population compared to the population in the US.

To elucidate the colonization scenario of *Aedes japonicus japonicus* in Europe seven microsatellite loci were studied in 106 individuals sampled in Germany and Switzerland in 2012. The probable sources of the European populations were calculated by using North American and Japanese samples as potential sources. On the population level the most probable source for all European samples appeared to be the Japanese samples. Therfore the WNV resistance of German *Aedes japonicus japonicus* might be due to genetically differentiated population.

EFFECTS OF LANDSCAPE AND MOSQUITO COMMUNITY ON WEST NILE VIRUS INCIDENCE IN WILD BIRDS

M. Ferraguti[1], J. Martínez-de la Puente[1], D. Roiz[2,1], S. Ruiz[3], R. Soriguer[4], J. Figuerola[1]

[1]Ecología De Humedales, Estación Biológica De Doñana (Ebd Csic), Sevilla, Spain, [2]Infectious Diseases and Vectors: Ecology, Genetics, Evolution and Control, Institut De Recherchepour Le Développement, Montpellier, France, [3]Servicio De Control De Mosquitos, Diputación De Huelva, Huelva, Spain, [4]Departamento De Etología Y Conservación De La Biodiversidad, Estación Biológica De Doñana (Ebd Csic), Sevilla, Spain

The distribution and incidence of many zoonotic diseases has increased in the last decades and most of them are transmitted by arthropod-vectors. Despite it has been suggested that biodiversity may affect pathogen transmission and disease incidence, little is known on how anthropogenic landscape transformation may affect pathogen transmission. In this study, we evaluated how human land use affect biodiversity patterns including vector community composition and abundance, and how these factors could affect the transmission of the flavivirus West Nile virus (WNV). WNV is a mosquito-borne disease for which birds serve as the primary reservoir hosts; but sometimes it could infect humans and horses causing disease. We sampled mosquitoes and wild house sparrows in 45 localities grouped in trios (15 urban, 15 rural and 15 natural) from Southern Spain. We analysed by ELISA the presence of WNV antibodies in 2392 house sparrows (Passer domesticus). Overall, the prevalence of WNV antibodies in house sparrows was low (2%). The age of birds and landscape use significantly affected the presence of antibodies in house sparrows. Older birds and birds from both natural (2.3%) and rural (2.7%) areas showed a higher prevalence of WNV antibodies than birds from urban areas (0.9%). Mosquito community composition and the abundance of potential WNV vectors differed among urban, rural and natural areas. Moreover, the prevalence of WNV was higher in those localities with the higher mosquito richness and where the abundance of non-competent vectors of WNV was lower. These results suggest that anthropogenic changes in landscape use and their subsequent effects on biological diversity may strongly influence the transmission dynamic of WNV.

FERAK VIRUS AND JONCHET VIRUS REPRESENT TWO NOVEL LINEAGES OF INSECT-RESTRICTED BUNYAVIRUSES

M. Marklewitz[1], F. Zirkel[1], A. Kurth[2], C. Drosten[1], S. Junglen[1]

[1]University of Bonn Medical Centre, Institute of Virology, Bonn, Germany, [2]Robert Koch Institute, Center for Biological Threads and Special Pathogens, Berlin, Germany

All known bunyavirus genera except hantaviruses are transmitted to their vertebrate hosts by arthropod vectors. Recently, two novel clades of putatively insect-restricted bunyaviruses were discovered (1,2), triggering new ideas regarding the origins of bunyaviruses. Here we describe two additional novel mosquito-associated bunyaviruses, designated Ferak virus (FERV) and Jonchet virus (JONV), which branch from a deep common node in sister relationship to the superclade orthobunyaviruses, tospoviruses and hantaviruses. FERV and JONV replicated in insect- but not in vertebrate cells. FERV virions diameter was 60-120 nm. JONV had two morphologies, one with tubular virions of 60 nm diameter and another with spheres of about 80 nm. RNA segment sizes were 1.5 kb, 4.3 kb, and of 6.9 kb for FERV and 1.7 kb, 5.4 kb, and of 6.9 kb for JONV. Pairwise identities of FERV and JONV were 23.8%, 10.3%, and 22.1% for L, M, and S segments, respectively. RdRp protein pairwise identities with bunyaviruses or tenuiviruses were below 15%. No significant similarity to any viral or cellular protein could be detected for the S and M segments. The lengths of conserved genome terminal elements were 11 nt for FERV (5'-AGUAGUAAACA) and 7 nt for JONV (5'-AGUAGUA). Evidence for the formation of mRNA and vRNA during virus replication was obtained for both viruses by northern blotting. Viral mRNAs showed variable 5'-terminal nontemplated sequences of 6 to 23 nt, suggestive of cap snatching activity. Both viruses encoded a non-canonical second ORF on the S segment. Expression of this putative NSs protein was confirmed by SDS-PAGE and MALDI-TOF. The genetic distance between JONV and FERV corresponded to that between the orthobunyaviruses and the recently described Herbert virus, or between the phleboviruses and the recently described Gouleako virus. Along with the different morphologies, segment sizes, and composition of genomic termini this suggests the putative presence of two novel Bunyaviridae genera. These findings highlight the diversity of bunyaviruses and provide further support to the idea that bunyaviruses might have evolved from arthropod associated viruses.

1. Marklewitz M., Handrick S., Grasse W., Kurth A., Lukashev A., Drosten C., Ellerbrok H., Leendertz F.H., Pauli G., Junglen S. (2011). Gouleako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family Bunyaviridae. Journal of Virology 85: 9227-9234.

2. Marklewitz M., Zirkel F., Rwego I.B., Heidemann H., Trippner P., Kurth A., Kallies R., Briese T., Lipkin W.I., Drosten C., Gillespie T.R., Junglen S. (2013). Discovery of a Unique Novel Clade of Mosquito-Associated Bunyaviruses. Journal of Virology 87: 12850-12865.

GLOBAL CHANGES AND WILDLIFE ZOONOTIC DISEASES EMERGENCE: THE CASE OF TICK-BORNE ENCEPHALITIS (TBE)

A. Rizzoli, D. Arnoldi, L. Bolzoni, F. Cagnacci, H.C. Hauffe, M. Neteler, C. Rossi, V. Tagliapietra, R. Rosà

Biodiversity and Molecular Ecology, Research and Innovation Centre Fondazione Edmund Mach, San Michele All'adige (Tn), Italy

Across the globe, tick borne zoonotic infections are emerging in new regions or re-emerging within endemic sites creating an increasing concern for public health, food security and biodiversity conservation worldwide. Of all the known zoonotic tick borne diseases, tick borne encephalitis caused by TBE virus (TBEV) is the most common viral disease transmitted to humans in Europe other than in central and eastern Asia by ticks of the family Ixodidae. It is now endemic in 27 European countries, and been declared an international public health threat.

The emergence of tick borne encephalitis is the consequence of several processes, spanning from changes in the exposure of humans to the infected ticks to changes in the bio-ecological condition affecting the TBE virus circulation within its natural cycles. We analyzed the pattern of TBE emergence in northern Italy combining eco-epidemiological studies with long term monitoring and extensive surveys on wildlife. Major drivers of disease emergence were identified in changes in forest structure and productivity other than in wildlife management practices. These changes results in variation of the pattern of tick infestation on rodents and TBEV circulation potential. However, although significant progress have been made in our understanding of TBEV ecology, several other factors need a better understanding to improve our ability to predict how the risk of TBE infection would change in the near future under a global change scenario.

This study was partially funded by EU grant FP7-261504 EDENext (www.edenext.eu).

NOVEL DIVERGENT VIRUSES DISCOVERED IN MOSQUITOES CAPTURED FROM DIFFERENT COUNTRIES OF EUROPE AND SENEGAL

A. Vázquez[1], M. Wiley[2], L. Cuevas[3], L. Herrero[1], S. Ruiz[4], M. Diallo[5], M. Grisenti[6], E. Pérez-Pastrana[3], L. Orshan[7], G. Fall[5], R.C. Soriguer[8], A. Rizzoli[6], H. Bin[9], N. Nowotny[10], G. Palacios[2], A. Tenorio[1], M.P. Sánchez-Seco[1]

[1]Laboratory of Arbovirus and Viral Infectious Diseases, Institute of Health Carlos Iii, Majadahonda, Spain, [2]United States Army Medical Research Institute for Infectious Diseases, Usamriid, Ft Detrick, United States, [3]Department of Electron Microscopy, Institute of Health Carlos Iii, Majadahonda, Spain, [4]Servicio Control Mosquitos, Diputacion Provincial De Huelva, Huelva, Spain, [5]Unité Des Arbovirus Et Virus De Fièvres Hémorragiques, Institut Pasteur De Dakar, Dakar, Senegal, [6]Fondazione Edmund Mach, Istituto Agrario Di San Michele All'adige, Trento, Italy, [7]Central Entomology Laboratory, Ministry of Health, Jerusalem, Israel, [8]Ebd, Csic, Seville, Spain, [9]National Center for Zoonotic Viruses, Laboratory of Environmental Virology, Tel Hashomer, Spain, [10]Department of Microbiology and Immunology, University of Veterinary Medicine, Vienna, Austria

In recent years, several researches have demonstrated the existence of a vast diversity of previously undiscovered viruses in the natural environment. During the past decade, a large number of insect-specific flaviviruses, which replicate in mosquito cells but not in vertebrate cells, have been discovered in several mosquito species. Recently, and thanks to deep sequencing technologies, a variety of other novel viruses have been isolated from pools of field-collected mosquitoes, such as rhabdoviruses, bunyaviruses, alphaviruses, nidoviruses, and reoviruses and/or negeviruses, suggesting that these types of agents are quite common in mosquitoes in nature. It is clear that viral transmission and maintenance cycles in nature are likely to be significantly more complex and taxonomically diverse than previously expected.

In this communication, we report the isolation and identification of new viruses belonging to several families of viruses detected in mosquitoes of several species of the genus *Culex* and *Aedes* from Spain, Italy, Israel and Senegal. We conducted systematic virus isolation in C636 cells from mosquito pools. When cythopatic effect was detected, isolates were analyzed by electron microscopy and partial or complete sequence was obtained by high throughput sequencing Systems.

We detected and obtained nearly full-genome sequence for potential new viruses belonging to the new taxon negevirus and the families *Rhabdoviridae*, *Reoviridae*, *Parvoviridae*, *Mesoniviridae*, *Birnaviridae*, *Bunyaviridae*. Further studies are needed in order to answer questions concerning the nature of these new viruses and possible interactions with other viruses.

Based on their wide geographic distribution, the limited sampling that has been done to date for these mosquito-specific viruses, and their broad species host range in mosquitoes, it seems likely that the insect-specific viruses are very common in mosquito populations worldwide. Their potential biological significance and effect of these viruses on mosquito vector competence with pathogenic viruses in nature is also discussed. Vector ecology may contribute to understand risk of zoonotic virus outbreaks.

RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY AGAINST *TOXOPLASMA GONDII*: RESULTS FROM THE FIRST REPRESENTATIVE SEROSURVEY OF ADULTS IN GERMANY

H. Wilking[1], M. Thamm[2], A. Aebischer[3], K. Stark[1], F. Seeber[3]

[1]Department for Gastrointestinal Infections, Zoonoses and Tropical Infections, Robert Koch Institute, Berlin, Germany, [2]Central Epidemiological Laboratory, Robert Koch Institute, Berlin, Germany, [3]Department for Mycotic and Parasitic Agents and Mycobacteria, Robert Koch Institute, Berlin, Germany

Toxoplasmosis, caused by *Toxoplasma* (*T.*) *gondii*, is a zoonotic infection contracted by contaminated food or contact to infested cat faeces. Primary infection during pregnancy may lead to malformations in neonates. Immunocompromised persons may experience manifest disease. Due to its potential severity it is considered a foodborne infection of significant public health concern. Germany is regarded an endemic area; however, data on the extent of endemicity and characteristics associated with seropositivity are scarce. Our objectives were to conduct a representative serosurvey among adults in Germany to assess the seroprevalence of *T. gondii* and to identify associated factors.

Sera from a nationwide representative survey of adults (DEGS) were tested by a commercial enzyme-linked fluorescence assay (ELFA) for anti-toxoplasma IgG-antibodies. Interviews data provided additional data. Multivariable logistic regression used sampling weights and accounted for survey design cluster effects. A catalytic model was used to estimate incidence of seroconversion.

Out of 6,663 individuals, 3,602 were seropositive. Seroprevalence increased from 20.0% (95%-CI:17.1%-23.1%) in the 18-29 age-group to 76.8% (95%-CI:72.7%-80.5%) in the 70-79 age-group. Increase with age was more pronounced in Eastern Germany. Male gender (OR:1.8; 95%-CI:1.1-2.9), keeping cats (OR:1.27; 95%-CI:1.06-1.51) and BMI≥40 (OR:2.6; 95%-CI:1.6-4.2) were independent risk factors for seropositivity; vegetarian status was negatively associated with seropositivity (OR:0.6; 95%-CI:0.4-1.0) as was a high socio economic status (OR:0.7; 95%-CI:0.6-0.9). As output of the model, 1,520 of 100,000 seronegative adults and 1,161 of 100,000 women aged 18-49 seroconvert. Extrapolated to the German population, this implies 4,370 annual seroconversions during pregnancies.

Our study demonstrates significant seroprevalence of *T. gondii* IgG, especially in older residents of Eastern Germany. The latter finding may be explained by a particular risk in this group or alternatively by a birth cohort effect of previous higher risk in East Germany. Variations in eating habits and cat ownership apparently influence seroprevalence.

Seropositivity is not equivalent with a history of clinical manifestation, but disease burden and seroprevalence are likely to be correlated. Toxoplasmosis is hard to combat and often neglected in public health programs targeting foodborne disease. Medical doctors and public health authorities should be aware. Food hygiene standards regarding *T. gondii* should be kept high.

SUCCESS IN DIVERSITY - INSIGHTS INTO THE POPULATION STRUCTURE OF *E. COLI*

T. Semmler[1], T.R. Connor[2,3], D.J. Pickard[2], R.A. Kingsley[2], N. Ahmed[4], C. Ewers[5], G. Dougan[2], N.R. Thomson[2], J.Corrander[6], L.H. Wieler[1]

[1]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [2]Pathogen Genomics, Wellcome Trust Sanger Institute, Cambridge Cb10 1 Sa, United Kingdom, [3]Cardiff University School of Biosciences, Cardiff University, Cardiff Cf10 3 Ax, United Kingdom, [4]Pathogen Biology Laboratory, Department of Biotechnology and Bioinformatics, University of Hyderabad, Hyderabad, India, [5]Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Giessen, Germany, [6]Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland

Escherichia (E.) coli is a highly diverse species, resulting in a quite complex population structure. The mixture of commensal and pathogenic strains is constantly evolving by genomic events like recombination, exchange of genomic regions via plasmids or phages as well as niche adaptation processes. A first step to define the genetic structure of *E. coli* was performed by creating a set of 72 strains from different hosts and locations, the so called ECOR collection by multilocus enzyme electrophoresis in 1983. We now know that this collection does not represent the whole *E. coli*population. To get insight into the whole population, we extended the dataset by whole genome analyses of more than 2.000 strains, choosing 420 of these genomes based on their belonging to one of more than 3.000 MLST profiles covering the population including the original ECOR strains. The set also includes "2nd population" strains which are already suspected to form a new species beside *E. coli*, as well as *E. albertii* and *E. fergusonii* as outgroups. The whole set was completely sequenced on Illumina HiSeq. Based on the maximum common genome (MCG), which contains the core genome of the analyzed *E. coli* strains, and was defined to achieve the highest possible phylogenetic resolution, we performed various analyses like Maximum Likelihood Phylogeny, Bayesian Analysis of Population Structure and Bayesian modeling of recombination events. The results show a clear clustering of the population into 14 groups which were further analyzed and compared, also considering the accessory genome of the strains which is responsible for the diversity within the *E.coli* population.

EARLY WARNING OF WEST NILE VIRUS MOSQUITO VECTOR: CLIMATE AND LAND USE MODELS SUCCESSFULLY EXPLAIN PHENOLOGY AND ABUNDANCE OF CULEX PIPIENS MOSQUITOES IN NORTH-WESTERN ITALY

R. Rosà[1], G. Marini[1], L. Bolzoni[2], M. Neteler[1], M. Metz[1], L. Delucchi[1], E.A. Chadwick[3], L. Balbo[4], A. Mosca[4], M. Giacobini[5], L. Bertolotti[5], A. Rizzoli[1]

[1]Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, San Michele All'adige (Tn), Italy, [2]Sezione Di Parma, Istituto Zooprofilattico Sperimentale Della Lombardia E Dell'emilia Romagna, Parma, Italy, [3]School of Biosciences, Cardiff University, Cardiff, United Kingdom, [4]Ufficio Lotta Alle Zanzare, Istituto Per Le Piante Da Legno E L'ambiente Ipla s.p.a, Torino, Italy, [5]Dipartimento Di Scienze Veterinarie, Università Degli Studi Di Torino, Torino, Italy

West Nile Virus (WNV) is an emerging global health threat. Transmission risk is strongly related to the abundance of mosquito vectors, typically *Culex pipiens* in Europe. Early-warning predictors of mosquito population dynamics would therefore help guide entomological surveillance and thereby facilitate early warnings of transmission risk. We analysed an 11-year time series (2001 to 2011) of *Cx. pipiens* mosquito captures from the Piedmont region of north-western Italy to determine the principal drivers of mosquito population dynamics. Linear mixed models were implemented to examine the relationship between *Cx. pipiens* population dynamics and environmental predictors including temperature, precipitation, Normalized Difference Water Index (NDWI) and the proximity of mosquito traps to urban areas and rice fields. Warm temperatures early in the year were associated with an earlier start to the mosquito season and increased season length, and later in the year, with decreased abundance. Early precipitation delayed the start and shortened the length of the mosquito season, but increased total abundance. Conversely, precipitation later in the year was associated with a longer season. Finally, higher NDWI early in the year was associated with an earlier start to the season and increased season length, but was not associated with abundance. Proximity to rice fields predicted higher total abundance when included in some models, but was not a significant predictor of phenology. Proximity to urban areas was not a significant predictor in any of our models. Climate data collected early in the year, in conjunction with local land use, can be used to provide early warning of both the timing and magnitude of mosquito outbreaks. This potentially allows targeted mosquito control measures to be implemented, with implications for prevention and control of West Nile Virus and other mosquito borne diseases.

DISENTANGLING THE ECOLOGICAL CONDITIONS AFFECTING WEST NILE VIRUS HAZARD IN THE OLD WORLD

M. Marcantonio, A. Rizzoli, M. Metz, R. Rosà, G. Marini, E. Chadwick, M. Neteler

Department of Biodiversity and Molecular Ecology, Fondazione Edmund Mach (Fem), San Michele All'adige, Italy

West Nile Virus (WNV) is a globally spreading Flavivirus found in all continent except Antarctica. It is recognised as a pathogen of medical and veterinary importance although in Europe it is not considered a major public health threat. However, the rapid change in the epidemiological pattern with re-emergence, introduction and spread into new territories since 2008 is of raising concern. Both prevention of WNV infection and the planning of vector suppression campaigns could benefit from early prediction of changes in the annual hazard. However, the identification of the biotic and abiotic conditions favouring WNV human transmission from endemic circulation is challenging due to the complexity of the virus transmission cycle. Furthermore, there is a lack of comprehensive epidemiological data since human and animal cases are under-reported.
We combined annual WNV epidemiological data collected by the European Centre of Disease Control (ECDC) across Europe and neighbouring countries in 2010, 2011 and 2012 with a number of environmental, biological and landscape factors. Remote Sensing and geographic information systems were used to collect high resolution environmental data, including temperature, precipitation, indices of water and vegetation, conservation status, land use, landscape structure, and human population density within areas of WNV circulation. We employed multi-model inference to gain a consensus from multiple linear mixed models predicting WNV incidence in humans, used as proxy of the hazard, at a scale of NUTS3/GAUL1 administrative units.
High precipitation in late winter/early spring, high summer temperatures, summer drought, and a high percentage of populated forest and irrigated croplands were identified by our model as predictors of WNV hazard in Europe, Western Asia and Northern Africa.
The integration of GIS, remote sensing and modelling tools with epidemiological data is a powerful approach to identify the biotic and abiotic conditions related to changes in the annual WNV hazard at continental scale. The development of reliable correlative models, able to connect ecological conditions with WNV hazard, is key to facilitate the preparedness of public health bodies to properly organise surveillance and control of WNV.

This includes also the speed up of diagnosis to implement blood and organ safety regulations.

This work was partially funded by the 7th Framework Programme European West Nile collaborative research project (Grant agreement n. 261391; www.eurowestnile.org) and by the Autonomous Province of Trento project LExEM (www.lexem.eu).

AN EPIDEMIOLOGICAL STUDY OF CANINE ECHINOCOCCOSIS AND LIVESTOCK HYDATIDOSIS IN SUDAN

N. Abass[1], M. Herzig[1], G. Boubaker[2], A.A. Elfadil[1], F.J. Conraths[1]

[1]Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald Isle of Riems, Germany, [2]Institute of Parasitology, University of Bern, Bern, Swaziland

A total of 1.991 domestic ruminants (535 sheep, 291 goats, 735 cattle, 430 camels) slaughtered in various abattoirs in the Sudan between July 2011 to January 2012 were examined for the presence of *Echinococcus granulosus* hydatid cysts. The prevalence of cystic echinococcosis (CE) was 25.3% (109/430, 95% CI 21.30-29.74%) in camels, 1.4% (11/735, 95% CI 0.74-2.67%) in cattle, 0.3% in sheep (2/535, 95% CI 0.04-1.34%) and 0.3% (1/291, 95% CI 0.00-1.90%) in goats. Of the total 98 cysts recovered from camels, 48(48.9%) fertile, 30 (30.6%) sterile and 20 (20.4%) were calcified and of the total 15 cysts from cattle, 12(80%), 2(13.3%)and 1(6.6%) were fertile, sterile and calcified, respectively . 101 camels had hydatid cysts only in the lungs (92.6%), 3 only in the liver (2.7%) and 5 camels had cysts both in the liver and in lungs. Most of the cysts were recovered from the lungs of cattle (72.7%) and some from the liver of cows (27.2%). Molecular analysis of both sub-multiplex PCR (Sub-mPCR) and sequencing of the mitochondrial marker coxI performed on cyst material from camels and cattle demonstrated that the study animals were infected with *E. granulosus* -camel strain- and *E. ortleppi*. Hydatid cyst prevalence was significantly higher in camels more than 3 years old compared with those aged 3 or less, in female camel compared with male camel (21.9% vs 2.8%). In the other hand, the prevalence of the disease in cattle was higher in Western Sudan (81.8%) than in Eastern Sudan (18.2%). In order to determine the prevalence and risk factors for canine echinococcosis in different areas in the country, 143 dogs (63 domestic and 80 stray dogs) were examined for *E. granulosus* infection by combined flotation and sedimentation followed by mPCR of mitochondrial genes for positive taeniid eggs samples. 33 dogs (23.07 %) were found harboring taeniid eggs. *E. granulosus* infection was detected in the feces of 56.5% (13/23, 95% CI 34.5-76.8%) and three samples 23.07% (3/13, 95% CI 5-53.8%) revealed co-infection with *E. granulosus* and *Taenia* spp. This study confirms that camels play an important role in the life cycle of *E. granulosus* in the Sudan.

THE POTENTIAL FOR RESPIRATORY DROPLET–TRANSMISSIBLE A/H5N1 INFLUENZA VIRUS TO EVOLVE IN A MAMMALIAN HOST

J.M. Fonville[1], C. Russell[2], A. Brown[3], D. Burke[1], D. Smith[4], S. James[1],S. Herfst[5], S. van Boheemen[5], M. Linster[5], E. Schrauwen[5], L. Katzelnick[1],A. Mosterin[1], T. Kuiken[5], E. Maher[6], G. Neumann[6], A. Osterhaus[5],Y. Kawaoka[6], R. Fouchier[5], D. Smith[1]

[1]Department of Zoology, University of Cambridge, Cambridge, United Kingdom, [2]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, [3]Medical Research Council Laboratory of Molecular Biology, University of Cambridge, Cambridge, United Kingdom, [4]Fogarty International Center, National Institutes of Health, Bethesda, United States, [5]Department of Virology, Erasmus Medical Centre, Rotterdam, Netherlands, [6]Department of Pathobiological Sciences, University of Wisconsin, Madison, United States

Avian A/H5N1 influenza viruses pose a pandemic threat. As few as five amino acid substitutions, or four with reassortment, might be sufficient for mammal-to-mammal transmission through respiratory droplets. From surveillance data, we found that two of these substitutions are common in A/H5N1 viruses, and thus, some viruses might require only three additional substitutions to become transmissible via respiratory droplets between mammals. We used a mathematical model of within-host virus evolution to study factors that could increase and decrease the probability of the remaining substitutions evolving after the virus has infected a mammalian host: random mutation, positive selection, long infection, alternate functionally equivalent substitutions, and transmission of partially adapted viruses as a proportion of the within-host diversity both in the avian-mammal and the mammal-mammal transmission events as well as an effective immune response, deleterious substitutions, and order-dependence in the acquisition of substitutions. Results will be presented that investigate the influence of these factors for starting viruses differing in the number of mutations that separates them from a respiratory droplet–transmissible A/H5N1 virus—viruses that require five, four, three, two, or one mutations at specific positions in the virus HA, reflecting that zero, one, two, three, or four of the mutations are already present in the avian population and thus are present at the start of the infection in mammals. From our analyses, it becomes clear that these mechanisms of acquiring mutations, combined with the presence of some of these substitutions in circulating strains, make a virus evolving in nature a potentially serious threat. These results highlight critical areas in which more data are needed for assessing, and potentially averting, this threat.

CLINICAL COURSE OF INFECTION AND TISSUE TROPISM OF HEPATITIS C VIRUS-LIKE HEPACIVIRUSES IN HORSES

S. Walter[1], S. Pfaender[1], J.M.V. Cavalleri[2], J. Doerrbecker[1], B. Campana[3], R.J.P. Brown[1], P.D. Burbelo[4], A. Postel[5], K. Hahn[6], A. Kusuma[1], N. Riebesehl[1], W. Baumgärtner[6], P. Becher[5], M.H. Heim[3], T. Pietschmann[1], K. Feige[2], E. Steinmann[1]

[1]Experimental Virology, Twincore, Hannover, Germany, [2]Clinic for Horses, University of Veterinary Medicine Hannover, Hannover, Germany, [3]Department of Biomedicine, University of Basel and Division of Gastroenterology and Hepatology, University Hospital Basel, Basel, Switzerland, [4]Dental Clinical Research Core, National Institute of Dental Craniofacial Research, National Institutes of Health, Bethesda, Maryland, United States, [5]Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany, [6]Department of Pathology, University of Veterinary Medicine Hannover, Hannover, Germany

Hepatitis C virus (HCV) has a very narrow species- and tissue tropism and efficiently replicates in the liver of humans and our closest relative, the chimpanzee. Recently, several studies reported some close relatives of HCV in different host species. Among these the non-primate hepaciviruses (NPHV) observed in horses, are the closest relatives of HCV described so far. In this study, we analyzed the prevalence of NPHV in northern Germany and characterized the clinical course of infection and viral tissue tropism to explore the relevance of HCV-related horse viruses as model for HCV infection and pathogenesis. We found that approximately 35.5 % of 445 horses were seropositive for antibodies against NPHV and about 2.5 % carried viral RNA. Liver function analyses revealed no indication of hepatic impairment in 7 of 11 horses. However, serum γ–glutamyl transferase (GGT) concentrations were mildly elevated in three horses and one horse displayed even highly elevated GGT levels. Characterization of an isolated equine cohort showed that 8 % of the horses were positive for NPHV RNA. In the course of a longitudinal follow up of this chort we observed that NPHV infection was cleared in individual horses with simultaneous induction of NS3-specific antibodies and concommittant elevation of serum levels of liver specific enzymes indicative of hepatic inflammation. In other individual horses chronic infections were observed with presence of both viral RNA and NS3-specific antibodies for over 6 months. To determine viral tissue tropism, we analyzed different organs and tissues of one NPHV positive horse and detected abundant NPHV RNA in the liver and spleen. To investigate a possible trans-species transmission of NPHV to humans, 172 horse veterinarians were tested for occupational exposure to NPHV. We found one individual with detectable antibodies against NPHV NS3 and one person with anti-E1E2 NPHV antibodies, but no viral NPHV RNA in the serum. In summary, these data demonstrate a high prevalence of NPHV in northern Germany with a low risk of trans-species transmission to humans. Similar to HCV in humans, we could demonstrate acute and chronic infections in horses with tissue-tropism for the horse liver.

SALMONELLA CO-OPTS FOR CMA FOR ITS INTRACELLULAR GROWTH AND SURVIVAL

V. Singh, J. Finke, P. Schwerk, K. Tedin

Institute of Microbiology and Epizootics, Centre for Infection Medicine, Freie University, Berlin, Germany

Salmonellosis is one of the most common food borne diseases in humans and presents a major public health and economic burden worldwide. *Salmonella* serovars are facultative intracellular pathogens which are invasive for host cells where they establish an intracellular, membrane-bound replicative niche known as the *Salmonella*-containing vacuole (SCV).
The SCV has been regarded as a nutrient deprived compartment. However, despite apparent nutrient limitation within the SCV, *Salmonella* is still able to replicate in the SCV. Here, we provide evidence for a unique mechanism whereby intracellular *Salmonella* gains access to the host cell cytosol from within its membrane-bound compartment to acquire nutrients. Our study shows that*Salmonella* Typhimurium acquires small peptides by co-opting the host cell chaperone mediated autophagy (CMA)-dependent cytosolic protein turnover pathway. CMA is a selective host cell protein turnover pathway active in all cell types and w is involved in the transport of cytosolic proteins into lysosomes for degradation. An estimated 30% of all cytosolic proteins are turned over through this mechanism. Here we show for both intracellular *Salmonella* and in purified SCVs that the SCV is associated with the key components of the CMA pathway, and inhibitors of CMA affect the intracellular growth of peptide-dependent mutants of *Salmonella*. Furthermore, we show that acquisition of the key CMA components is selective, with recruitment of only one isoform of one host protein component, and exclusion of other lysosomal proteins.
We suggest our results may provide an explanation for relapse infections and shedding of *Salmonella* by both human and animal long-term carriers

THE TICK-BORNE PATHOGEN *CANDIDATUS NEOEHRLICHA MIKURENSIS* IN *IXODES RICINUS* AND NATURAL VERTEBRATE HOSTS IN SOUTHERN SWEDEN

M. Andersson

Biology Department, Lund University, Lund, Sweden

The bacterium *Candidatus Neoehrlichia mikurensis* has recently been recognized as a human pathogen, following its discovery in human patients from several European countries. It appears to be one of the most common pathogens in Ixodes ricinus in large parts of Europe and can be found in several different rodent species that are likely to be reservoir hosts. Recently, seven human cases were reported from China, showing a widespread distribution of *Candidatus N. mikurensis* across Europe and Asia. We developed a qPCR assay for identification and quantification of *Candidatus N. mikurensis* DNA, and determined the prevalence in questing *I. ricinus* in southern Sweden to be 6.0% (n=949). The prevalence in bank voles (*Myodes glareolus*), one of the most abundant rodent species in northern Europe, was 18%, indicating that bank voles could serve as important reservoir hosts, if it turns out that they are competent hosts for this bacterium. The co-infection rate with *Borrelia afzelii* was significantly higher than expected from random co-occurrence, both in *I. ricinus* and in bank voles, possibly reflecting positive interactions between these pathogens or a general susceptibility in certain individuals in the vole population. The epidemiology in the bank voles differed quite dramatically between *B. afzelii* and *Candidatus N. mikurensis*. The prevalence of *Candidatus N. mikurensis* increased radically over the summer season, resulting in that almost half the rodent population was infected at the end of the summer. In contrast, the prevalence of *B. afzelii* varied less dramatically over the transmission season. In conclusion, *Candidatus N. mikurensis* DNA frequently occurs in I. ricinus and rodents in southern Sweden, indicating that the risk for humans to come into contact with infected ticks is substantial.

CULEX PIPIENS AND CULEX TORRENTIUM MOSQUITOES FROM GERMANY HAVE VECTOR COMPETENCE FOR WEST NILE VIRUS

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

[1]Molecular Entomology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Arbovirology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [3]Kabs e.v., German Mosquito Control Association, Waldsee, Germany, [4]Department of Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [5]German Centre for Infection Research, Partner Site Hamburg Lübeck Borstel (Bernhard Nocht Institute for Tropical Medicine), Hamburg, Germany, [6]Department of Tropical Medicine (Medical Entomology), Bundeswehr Hospital Hamburg, Hamburg, Germany

Background: West Nile virus (WNV), a flavivirus with an avian primary host, can pose an infection risk to Germany, since millions of birds migrate every year between West Nile endemic areas in south and southeast Europe to Germany. If competent vectors are present, introduction of WNV could establish an enzootic transmission cycle in Germany. Therefore, we addressed the risk of a WNV circulation through *Culex pipiens* and *Culex torrentium* mosquitoes, which represent two of the most abundant mosquito species in Germany.

Findings: *Culex pipiens* and *Culex torrentium* adult mosquitoes from Germany are both susceptible to WNV infection, with infection rates between 33 and 76% at 25°C, respectively. Infection rates were consistent over two subsequent seasons (2012 and 2013) and 82-96% of infected mosquitoes had disseminating infections. The ornithophilic species *Culex pipiens* biotype *pipiens* and *Culex torrentium* promoted WNV infection at 18°C with infection rates highly similar to those at 25°C (18°C 27-79%), whereas the anthropophilic species *Culex pipiens* biotype *molestus* did not support replication of WNV at low temperatures.

Conclusion: These findings indicate that a WNV enzootic infection cycle in Germany is possible. However, transmission of WNV to humans by *Culex pipiens* biotype *molestus* appears rather unlikely, since prolonged periods with temperatures of 25°C required for WNV replication in this anthropophilic species, are rarely being reached in Germany.

VIRUS DISCOVERY IN MOSQUITOES FROM THE NEOTROPICS

A. Kopp[1], A. Hübner[1], F. Zirkel[1], D. Hobelsberger[2], A. Estrada[3], T. Gillespie[4,5], C. Drosten[1], S. Junglen[1]

[1]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [2]Robert Koch Institute, Emerging Zoonoses, Berlin, Germany, [3]Estación De Biología Tropical Los Tuxtlas, Instituto De Biología, Universidad Nacional Autónoma De México, Mexico City, Mexico, [4]Department of Environmental Studies and Program in Population Biology, Ecology and Evolution, Emory University, Atlanta, United States, [5]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, United States

Arthropod-borne diseases are a significant burden for human health. New arboviruses often originate or emerge from tropical regions. Knowledge of virus diversity in tropical rainforests is crucial for emerging disease surveillance and forecasting.

In order to analyse arboviruses diversity in the neotropics, 3.491 mosquitoes were collected in and around Palenque National Park, Mexico. Pools of homogenized mosquitoes (n=371) were screened by generic PCR and cytopathic effect (CPE) assays on the mosquito cell line C6/36. Of the 264 tested pools, 71 induced CPE. In 18 pools a novel strain of Piura virus (unclassified) was detected. The genome of one isolate was completely sequenced by next generation sequencing. The Piura Palenque strain shared 95% pairwise nucleotide identity with Piura virus. No viral sequences were retrieved from the remaining 53 CPE positive pools suggesting the isolation of novel viruses.

Mosquito pools were tested for alphavirus, bunyavirus, mesonivirus, and rhabdovirus infections by PCR. No alphavirus was detected. Sequences distantly related to phleboviruses were detected in 109 individual mosquitoes. Maximal amino acid identity within the core polymerase domain (~1kb) was identified to Gouléako virus (54-58%). Phylogenetic analyses indicated grouping into seven distinct species belonging to four novel clades. Furthermore, a sequence fragment (~2kb) with 87% nucleotide identity to Wyeomyia virus (genus Orthobunyavirus) was identified in one pool. Two sequences (~2kb) with identities of 58-65% to the novel Herbert virus clade were found in two pools. One of these viruses branched from a deep node in basal relationship to orthobunyaviruses, the other formed a sister taxon to members of the Herbert virus clade. A putative novel mesonivirus in basal phylogenetic relationship to all known insect nidoviruses was detected. Maximal pairwise identity of 78% to Méno virus was identified for a 1.5 kb fragment.

Sequencing of a ~1kb fragment suggested that a putative novel rhabdovirus was found. Maximal nucleotide identities of 58% were identified to Beaumont virus and North Creek virus, both detected in Australian mosquitoes. In phylogenetic analyses these viruses formed a clade basal to the dipteran-mammal associated rhabdovirus (dimarhabdovirus) group.
These data emphasize that a high diversity of unknown viruses in tropical mosquitoes exists.

DETECTION OF NGARIVIRUS, A HIGHLY VIRULENT ORTHOBUNYAVIRUS IN SMALL RUMINANTS FROM MAURITANIA

M. Eiden[1], A. Vina-Rodriguez[1], B.O. El Mamy[2], K. Isselmou[2], U. Ziegler[1], D. Höper[3], S. Jäckel[1], A. Balkema-Buschmann[1], H. Unger[4], B. Doumbia[2], M.H. Groschup[1]

[1]Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald, Germany, [2]Centre National De L'elevage Et De Recherches Vétérinaires (Cnerv), Service De Pathologie Infectieuses, Nouakchott, Mauritania, [3]Friedrich-Loeffler-Institut, Institute for Diagnostic Virology, Greifswald Isle of Riems, Germany, [4]Iaea, Joint Fao/iaea Division, Vienna, Austria

Ngari virus (NRIV) is a single stranded RNA virus belonging to the family Bunyaviridae; genus Orthobunyavirus which evolved by genetic reassortment of Bunyamwera and Batai virus. In contrast to the low pathogenic progenitor viruses, NRIV harbors an elevated virulence and can cause acute hemorrhagic fevers in humans. Human cases were observed in Kenya, Tanzania and Somalia in the context of Rift Valley Fever (RVF) outbreaks.

In our study we have determined the RVF antibody status in a large number of animal sera that were collected during the RVF outbreak in Mauritania in year 2010. Sera were also screened by a SYBRgreen based pan-orthobunya-PCR to discover infections with other members of the Bunyaviridae family. Most interestingly by using this approach we detected NRIV genomes in two goat samples. Viruses could eventually be isolated from these sera and completely sequenced by next generation sequencing. The obtained sequence is the first ruminant derived NRIV sequence, as published sequences originated from human and mosquito sources. Interestingly, both NRIV positive animals carried also RVFV specific IgG antibodies and one of them even IgM antibodies indicating a possible co-infection of RVFV and NRIV.

The importance of mono-causal NRIV and of NRIV/RFVV co-infections for humans, livestock and wildlife animals in Africa remains unclear. Studies are also needed on mosquito vectors that are involved in the NRIV transmission cycle. Moreover, discriminatory molecular and serological surveys are essential to reveal the current prevalence of NRIV infection in humans and livestock in Mauritania and other African countries.

ANTI-IDIOTYPIC ANTIBODIES IN DIAGNOSIS OF OPISTHORCHIASIS

A. Bulashev[1], S. Serikova[1], S. Eskendirova[2]

[1]Faculty of Veterinary Medicine and Livestock Technology, Seifullin Kazakh Agro Technical University, Astana, Kazakhstan, [2]Laboratory of Immunochemistry and Immunodiagnosis, National Center for Biotechnology, Astana, Kazakhstan

In the system of Opisthorchiasis control the main role is given to the early diagnosis. Effectiveness of existing diagnostic methods of Opisthorchiasis (investigation of duodenal juice and coprological study) is rather low. Opisthorchiasis diagnosis can be also established on the basis of serological tests such as Radial Immunodiffusion and Indirect Hemagglutination Test. These tests have objectivity at an early the system of Opisthorchiasis control the main role is given to the early diagnosis. Effectiveness of existing diagnostic methods of Opisthorchiasis (investigation of stage, i.e. prior to eggs release and less effective in chronic stages of the disease. Furthermore, they are unsuitable for mass-screening assay.

In recent years highly sensitive immunological methods, including ELISA, are introducing into diagnostic practice. However, using in these tests multi-somatic antigens or Excretory-secretory antigen (ES-Ag) of trematodes does not provide the desired results, because positive reactions of patients with other helminthiases are observed. These facts can be explained by the presence of cross-reacting antigens among helminths and raise the need to search for specific antigens.

The aim of research was to develop express-tests for serological diagnosing Opisthorchiasis based on using Monoclonal antibodies (Mab) and Anti-idiotipic antibodies (AIab).

Sensitization of polystyrene wells with specific ES-Ag protein by means of MAb 4B3D9 gave ELISA-test high sensitivity in investigations of blood sera of patients suspected to infestation with Opisthorchiasis. Unfortunately, this test was not practical due to the difficulty of obtaining ES-Ag. In this regard with the help of hybridoma technique two hybrid strains 3H10A4 and 4H10D8 were obtained producing AIab to MAb against ES-Ag epitope in the protein structure with a molecular weight of 28 kD.

AIab as an "internal image" of Opisthorhis felineus ES-Ag initiated an immune response in a laboratory animals in the form of antibody formation. Antigenicity of anti-idiotypes was tested by indirect and sandwich ELISA in comparison with pathogen's ES-Ag using serum of experimentally infested dog. The results showed that anti-idiotypes by its diagnostic value are not inferior to natural parasite's antigen. Thus, AIab as an antigen can be used in developing test systems for serological diagnosis of Opisthorchosis.

DEVELOPMENT OF A CELL CULTURE SYSTEM FOR HEPATITIS E VIRUS

R. Johne[1], E. Trojnar[1], J. Reetz[1], P. Nickel[2], R.G. Ulrich[3], P. Machnowska[1], J. Sachsenroeder[1], J. Hofmann[4]

[1]Food Hygiene and Virology, Federal Institute for Risk Assessment, Berlin, Germany, [2]Department of Nephrology and Intensive Care, Charite Campus Virchow Klinikum, Berlin, Germany, [3]Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald Isle of Riems, Germany, [4]Institute for Virology, Charite Campus Mitte, Berlin, Germany

Hepatitis E is an increasingly reported zoonotic disease in Europe. A foodborne transmission of the causative agent, hepatitis E virus (HEV), is suspected. Studies on its stability and replication cycle as well as the development of antiviral substances and vaccines are hampered due to the lack of efficient and robust cell culture systems.
Here, the successful isolation of a HEV strain derived from a chronically infected transplant patient held under immunosuppressive therapy is described. A serum sample was inoculated onto the human lung carcinoma cell line A549 resulting in replication of the virus as shown by RT-qPCR. Genome analysis of the inoculated HEV strain indicated the closest relationship with a wild boar-derived genotype 3 strain, which was not capable of replication in A549 cells. An insertion of 186 nucleotides originating from the HEV ORF1 was identified in the hypervariable genome region of the patient strain, but not in the wild boar strain. A cell line continuously producing HEV particles was generated by passaging cells infected by the patient strain. Although the endpoint titers of the virus in the culture supernatant were relatively low (10^6 genome copies per ml), the produced virus could be repeatedly passaged in A549 cells, as shown by RT-qPCR, immunohistochemistry and electron microscopy, without a cytopathic effect.
The data indicate robust cell culture replication of an uncommon HEV strain. Recently, cell culture isolation of two other HEV strains carrying also insertions in their hypervariable regions, but originating from human ribosomal RNA genes, have been described by other groups. The findings indicate that efficient tissue culture adaptation of HEV is – by unknown reasons - influenced by the presence of an insertion in the hypervariable genome region. The cell culture system is currently used for testing virus stability after heat treatment and food processing, but may also be useful for analysis of the replication cycle of HEV and development of strategies for replication inhibition.

EVALUATION OF AN INDIRECT ELISA USING A TACHYZOITE SURFACE ANTIGEN SAG1 FOR DIAGNOSIS OF *TOXOPLASMA GONDII* INFECTION IN CATS

F. Hosseini, M. Hosseininejad

Veterinary Clinical Sciences, University of Shahrekord, Shahrekord, Islamic Republic Of Iran

Toxoplasma gondii infection is very common in cats throughout the world. Most cats are subclinically infected and potentially fatal clinical disease occurs in some of them. The aim of this study is to develop an indirect enzyme linked immunosorbent assay (ELISA) test using an affinity purified tachyzoite surface antigen (SAG1) to detect *T. gondii* infection in cats. Six sero-negative kittens were used in this study; four kittens received 104 *T. gondii* tachyzoites of NED strain (type III) and the remaining two were used as uninfected controls. Serum samples were collected within 41 days and were evaluated for anti- *T. gondii* antibodies using indirect fluorescent antibody test (IFAT) and ELISA method. IgG antibodies were detectable at least from eight days after tachyzoites inoculation and an increasing pattern in both serum ELISA indices (SIns) and IFAT titers were detected. SIns were significantly different in sera of cats presenting different IFAT titers. In order to evaluate the performance of ELISA to detect anti- *T. gondii* antibodies of naturally infected cats, serum samples were also collected from household and stray cats and evaluated in the same way. IFAT was regarded as the standard test and sensitivity and specifity of the ELISA to detect the infection in naturally infected kittens were analyzed using two-graph receiver operating characteristic (TG-ROC) analysis. An area under curve (AUC) of 0.996 revealed the test as a highly accurate test with relative sensitivity and specificity of 100 and 96% for a cut-off value of 0.10 for SIn.

MYCOBACTERIUM AVIUM SUBSP. *PARATUBERCULOSIS* SHEEP STRAIN JIII-386: SEQUENCING, ASSEMBLING, ANNOTATION, AND GENOME COMPARISON

P. Möbius, M. Hölzer, M. Felder, G. Nordsiek, M. Groth, H. Köhler, K. Reichwald, M. Platzer, M. Marz

Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Federal Institute for Animal Health, Jena, Germany

Mycobacterium avium subsp. paratuberculosis (MAP) - the etiologic agent of paratuberculosis - affects cattle, sheep and other ruminants. MAP was detected in environmental samples, in raw milk and isolated from man (Cattle-Type). The aetiologic role of MAP in human Crohn's disease is under discussion. The deciphering of phenotypic differences including virulence and host association observed between cattle and sheep strains (belonging to Type-II and I/III) by comparative genome analysis is hampered by the lack of a fully assembled MAP-Type-I/III strain genomic sequence.

MAP sheep strain JIII-386 (MAP-Type III) from a migrating herd in Germany was subjected to whole-genome shotgun sequencing, de novo assembled, and annotated by BacProt. NcRNAs were annotated by homology search of Rfam families using the GORAP pipeline. Additionally, a new full sequence of cattle isolate JII-1961 from Germany, published MAP-Type-II strains K10, MAP4, MAP-Type-III strain S397 (all from U.S.), MAP-Type-I strain CLIJ361 from Australia, and *M. a. subsp. hominissuis* strain MAH104 were used for comparison and assembly improvement of JIII-386. These genomes were also fully annotated by BacProt and results compared with NCBI annotation.

With JIII-386, the so far best assembled Type-I/III strain is presented here. Using two annotation programs, equal numbers of gene sequences were found, but also 10 % of genes only by either NCBI or BacProt. A new Shine-Dalgarno sequence motif was extracted, possibly conserved for Mycobacteria. Novel mycobacteria-specific proteins were searched. For the first time about 80 ncRNAs and Riboswitches were unveiled for MAP, numbers of which differ between MAP-Type-III and II (ASpks, G1, ykkC-III) but also between MAP types and MAH104. Some previously described differences between genomes of MAP-Type-I/III and II strains could be partially revised, two new Type-I/III specific large regions identified. Results of SNP analysis confirm the strong similarity of MAP-Type-II strains, and show higher diversity among MAP-Type I/III strains.

Enhancement of known strain diversity at genome level as achieved by providing two new MAP sequences and the use of two annotation programs unveiled new insights in MAP-Type specific gene regions and will help to decipher genes responsible for different host association and virulence of Type I, II, and III.

HYGIENISATION OF *MYCOBACTERIUM FORTUITUM* IN CATTLE MANURE THROUGH LACTIC ACID FERMENTATION

H.A. Scheinemann[1], M. Krüger[2]

[1]Gesellschaft Zur Förderung Von Medizin , Bio Und Umwelttechnologien E. V., Fachsektion Halle, Halle, Germany, [2]Veterinärmedizinische Fakultät Universität Leipzig, Inst. Für Bakteriologie Und Mykologie, Leipzig, Germany

Manure from animal farms and sewage sludge contain pathogens and opportunistic organisms in various concentrations depending on the health of the herds and human sources. Besides the presence of pathogens, these waste substances are excellent nutrient sources and constitute a preferred organic fertilizer. However, because of the pathogens, the risks of infection of animals or humans increase with the indiscriminate use of manure, especially liquid manure or sludge, for agriculture. This potential problem can increase with the global connectedness of animal herds fed imported feed grown on fields fertilized with local manures. Being one of the most common pathogens, *Mycobacteria* causes infectious diseases with the highest mortality rates worldwide. This presentation describes a simple, easy-to-use, low-tech hygienisation method, which conserves nutrients and does not require large investments in infrastructure. The proposed method uses the microbiotic shift during lactic acid fermentation of cow manure during which gram-negative bacteria, enterococci and yeasts were inactivated below the detection limit of 3 log10 cfu/g, while lactobacilli increased up to a thousand fold. Pathogens like *Salmonella, Listeria monocytogenes, Staphylococcus aureus, E. coli* EHEC O:157, vegetative *Clostridium perfringens* and ECBO-viruses were inactivated, as shown in a previous poster in 2013. This time, *Mycobacterium fortuitum* was tested and inactivated as well. This method might be an acceptable hygienisation method for developed as well as undeveloped countries, and could play a key role in public and animal health while safely closing the nutrient cycle by reducing the necessity of using energy-inefficient inorganic fertilizer for crop production.

ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR DETECTION OF ANTIBODIES TO THE NOVEL LLOVIU (FILOVIRUS) AND CRIMEAN CONGO HEMORRHAGIC FEVER VIRUSES LOCATED IN SPAIN.

E. Ramírez de Arellano[1], M. Ortiz[1], F. Lasala[1], P. López[1], M.J. Perteguer[1], L. Fernández[1], M. Ángel Habela[2], A. Estrada-Peña[3], G. Palacios[4], M.P. Sánchez-Seco[1], A. Tenorio[1], A. Negredo[1]

[1]Centro Nacional De Microbiología, Instituto De Salud Carlos Iii, Madrid, Spain, [2]Dpto. Parasitología, Facultad De Vaterinaria/ Universidad De Cáceres, Cáceres, Spain, [3]Dpto. Parasitología, Facultad De Veterinaria/ Universidad De Zaragoza, Zaragoza, Spain, [4]United State Army Medical Research Institute for Infectious Diseases, Usamrid, Ft Detrick, Washington, United States

A novel filovirus, named LLoviu virus (LLOV), was detected in bat carcasses in an episode of bat die-offs occurred in the Northern Spain in 2002. More recently, since 2011, two genetic variants of Crimea Congo Hemorrhagic Fever virus (CCFHV) included in one of the African genotypes (genotype 3) have been detected in ticks in a rural region of Western Spain suggesting a possible virus establishment. Human and veterinary surveillance using suitable diagnostic tools are needed in our country to ascertain possible implications for public health. The development of methods based on Enzyme–Linked Immunosorbent Assay (ELISA) could be very useful to carry out studies of seroprevalence in affected geographical areas.We have used the Gp2 glycoprotein of LLOV as the antigenic protein for the detection of IgG antibodies in the ELISA assay. The Gp2 has been expressed as a His-tagged recombinant protein in the baculovirus system. Insect cells were infected with the recombinant baculoviruses and the purified protein was determined by Western blotting. The Gp2 protein of LLOV was strongly expressed in insect cells. On the other hand, to develop an ELISA for detection of antibodies to CCFHV, two described immunogenic fragments of Nucleoprotein (NP) were selected as antigens. They were expressed as glutathione S-transferase-tagged recombinant proteins in the Escherichia coli system. These purified proteins are going to be used to detect IgG antibodies in collected human sera from risk populations in Spain. The developed tools will be used to evaluate the infection in humans for these hemorrhagic viruses in Spain.

INEFFICIENT CELLULAR TRANSPORT OF THE ATTACHMENT GLYCOPROTEIN OF THE AFRICAN HENIPAVIRUS M74 PLAYS A MAJOR ROLE FOR THE RESTRICTED FUNCTIONAL ACTIVITY

N. Krüger[1], M. Hoffmann[1], M.A. Müller[2], J.F. Drexler[2], C. Drosten[2], G. Herrler[1]

[1]Institute of Virology, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [2]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Bats are known to be the reservoir hosts for many zoonotic viruses. Flying foxes of the genus *Pteropus* that occur in South-East Asia have been reported to be the reservoir of the henipaviruses Hendra (HeV) and Nipah virus (NiV). These viruses can lead to severe and often fatal infections in humans, whereas infected bats do not show any symptoms. The detection of henipavirus RNA in spleen samples of African flying foxes (Drexler *et al.*, 2009) has shown that this group of viruses has a wider global distribution. The zoonotic potential of African henipaviruses is unknown.

We focused on the functional characterisation of the surface glycoproteins of the African Henipavirus BatPV/Eid_hel/GH-M74a/GHA/2009 (M74) which was isolated from an African flying fox of the species *Eidolon helvum*. We recently showed that the functional activity of the M74 fusion (F) and attachment glycoprotein (G) is restricted to chiropteran cells (Krüger *et al.*, 2013).

First, we addressed the question if differences in the expression or proteolytic cleavage of the M74-F can explain the restricted functional activity. Western Blot analysis revealed that the F protein was as efficiently expressed and cleaved in cell lines which do not support fusion and in chiropteran cells which support fusion. Next, we focused our studies on the M74-G. When we analysed the intracellular expression pattern of M74-G, co-localisation was obtained with a cellular compartment marker for the endoplasmatic reticulum (ER), but not with markers for the ER-Golgi-intermediate-compartment (ERGIC), the Golgi apparatus, or endosomes. Endoglycosidase digestion of the M74-G indicated that the protein does not contain detectable amounts of complex N-glycans. Surface biotinylation and flow-cytometry showed that the majority of the M74-G is expressed intracellularly and only minor amounts reach the cell surface, even in cell lines, which support cell-to-cell fusion after co-expression of M74-F and -G. However, the amount of surface expressed G protein was higher in chiropteran cells in comparison to the cells which do not support fusion.

Our results indicate that the transport of the M74-G protein from the ER to the cell surface is inefficient and explains the restricted functional activity of this henipavirus glycoprotein.

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) TREATMENT AMELIORATES TOXOPLASMA GONDII-INDUCED ENCEPHALITIS IN MICE

A. Parlog[1], L. Möhle[1], A. Biswas[1], D. Reglodi[2], M.M. Heimesaat[3], I.R. Dunay[1]

[1]Department for Microbiology and Hygiene, University of Magdeburg, Magdeburg, Germany, [2]Department of Anatomy, Pte Mta Lendület Pacap Research Team,, University of Pecs, Pecs, Hungary, [3]Department for Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany

Background: The intracellular parasite *Toxoplasma (T.) gondii* infects up to one third of the world population. The latent infection can reactivate in case of immunosuppression resulting in life-threatening encephalomyelitis. Current therapeutic regimens, however, exert major adverse effects. Furthermore, to date no current agent is able to completely eliminate the latent stage of the parasites, namely cysts, from the central nervous system (CNS). Hence, the demand for novel therapeutic drugs that reduce inflammation, repair neuronal degeneration and eliminate the chronic stage of parasites is urgent. Pituitary adenylate cyclase-activating polypeptide (PACAP) is well known to play crucial roles in immunity and inflammation. For the first time, we investigated the potential anti-inflammatory and immune-modulatory properties of PACAP in a murine parasite-induced encephalitis model.

Methodology/Principal Findings: Encephalitis was induced following intraperitoneal *T. gondii* infection (3 cysts, ME49 strain) four weeks before analysis. Synthetic PACAP38 was administered intraperitoneally (1.5 mg / kg body weight) every other day for 10 days starting at day 18 post infection (p.i.). On day 28 p.i., PACAP treated animals displayed reduced signs of intracerebral inflammation when compared to placebo treated controls as indicated by less inflammatory foci and fewer CD3+, F4/80+ and Caspase3+ cell numbers within the brain parenchyma. Importantly, PACAP treated mice exhibited significantly lower intracerebral IFN-gamma, IL-6 and IL-10 mRNA expression levels as compared to placebo controls. Moreover, a trend towards reduced *T. gondii*loads in the CNS upon PACAP treatment could be observed. Interestingly, intracerebral p75NTR mRNA expression levels were reduced in PACAP-treated group, suggesting involvement of the neurotrophin signaling pathway.

Conclusion/Significance: PACAP treatment ameliorates *T. gondii* induced inflammation in a murine model. These findings might provide new treatment options for Toxoplasma encephalitis.

FLU´S FIRST DEFENSE AGAINST IFN: VIRAL SUPPRESSORS OF TYPE I IFN RESPONSE ARE PREPACKAGED IN INFLUENZA A VIRUS VIRIONS

S. Liedmann[1], E.R. Hrincius[2], C. Guy[3], D. Anhlan[1], R. Dierkes[1], G. Wu[4], D.R. Green[3], T. Wolff[5], J.A. McCullers[6], S. Ludwig[1], C. Ehrhardt[1]

[1]Institute of Molecular Virology, University of Muenster, Muenster, Germany, [2]Department of Infectious Diseases, St. Jude Children´s Research Hospital, Memphis, United States, [3]Department of Immunology, St. Jude Children´s Research Hospital, Memphis, United States, [4]Department of Computational Biology, St. Jude Children´s Research Hospital, Memphis, United States, [5]Division of Influenza and Other Respiratory Viruses, Robert Koch Institute, Berlin, Germany, [6]Department of Infectious Diseases, St. Jude Children´s Research Hospital, Memphis, Germany

The type I interferon (IFN) response represents the first line of defense to invading pathogens. Internalized viral ribonucleoproteins (vRNPs) of influenza A viruses (IAV) provoke induction of an early IFN response by interacting with RIG-I, a cytosolic pathogen pattern receptor that is recruited to mitochondria.

We employed three-dimensional stochastic optical reconstruction microscopy (STORM) to visualize incoming influenza A virus (IAV) vRNPs as distinct helical structures associated with mitochondria. Co-localization with RIG-I and MAVS would suggest an early IFN response upon infection. However, no such response was detected. We observed that a distinct amino acid motif in the viral polymerases, PB1 and PA, suppresses early IFN induction. Mutation of this motif leads to reduced pathogenicity *in vivo*, while restoration increases it. Evolutionary dynamics in these sequences suggest that restoration of the motif, combined with viral reassortment can contribute to pandemic risks.

In summary, we identified PB1 and PA as new players in the type I IFN inhibitory strategy evolved by IAV filling the gap in knowledge regarding early suppression of antiviral immune responses. In contrast to the NS1 protein, PB1 and PA are already present when internalized vRNPs are sensed by RIG-I and are located in close proximity to the site of RIG-I activation. Therefor, the suppression of the immediate anti-viral response is „prepackaged" in IAV in the sequences of vRNP-associated polymerase proteins**.**

MOLECULAR BASIS FOR DISRUPTION OF E-CADHERIN ADHESION BY BOTULINUM NEUROTOXIN A COMPLEX

K. Lee[1], X. Zhong[2], S. Gu[1], A.M. Kruel[3], M.B. Dorner[4], K. Perry[5], A. Rummel[3], M. Dong[2], R. Jin[1]

[1]Department of Physiology and Biophysics, University of California, Irvine, Ca, United States, [2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston Southborough, Ma, United States, [3]Institut Für Toxikologie, Medizinische Hochschule Hannover, Hannover, Germany, [4]Centre for Biological Threats and Special Pathogens – Biological Toxins (Zbs3), Robert Koch Insitute, Berlin, Germany, [5]Ne Cat and Department of Chemistry and Chemical Biology, Cornell University, Argonne, Il, United States

How botulinum neurotoxins (BoNTs) cross the host intestinal epithelial barrier in foodborne botulism is poorly understood. Here, we present the crystal structure of a clostridial hemagglutinin (HA) complex of serotype BoNT/A bound to the cell adhesion protein E-cadherin at 2.4 Ångströms. The HA complex recognizes E-cadherin with high specificity involving extensive intermolecular interactions and also binds to carbohydrates on the cell surface. Binding of HA complex sequesters E-cadherin in the monomeric state thereby compromising the E-cadherin-mediated intercellular barrier and facilitating paracellular absorption of BoNT/A. We reconstituted the complete 14-subunit BoNT/A complex using recombinantly-produced components and demonstrated that abolishing either E-cadherin- or carbohydrate-binding of HA complex drastically reduces oral toxicity of BoNT/A complex *in vivo*. Together, these studies establish the molecular mechanism of how HAs contribute to the oral toxicity of BoNT/A.

NOVEL IMMUNOSTIMULATORY FLAGELLIN-LIKE PROTEIN FLAC IN CAMPYLOBACTER JEJUNI AND OTHER CAMPYLOBACTERALES

E. Leno[1], E. Gripp[1], S. Maurischat[2], B. Kaspers[3], K. Tedin[2], S. Klose[1], C. Josenhans[1]

[1]Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany, [2]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany, [3]Institute for Animal Physiology, Ludwig Maximillians Universität Munich, Munich, Germany

Campylobacter jejuni and *Campylobacter coli* are bacterial pathogens which colonise different hosts and preferentially cause acute intestinal disease such as diarrhea in humans, while they persist chronically in several different animal species including avian and mammalian hosts (1). Little is known about how *Campylobacter* ssp. interact with the innate immune systems of their hosts and with the major pattern recognition receptors (PRR) such as TLR and NOD receptors. It has been reported that *C. jejuni* is restricted in its ability to activate the innate immune system via TLR5 (2).

Methods and Results: In addition to the classical flagellin molecules, we found the unusual flagellin-like protein FlaC and potential orthologues to be conserved in nine different *Campylobacter*, three intestinal *Helicobacter* and one *Wolinella* species. FlaC is a secreted protein, not involved in motility. Its amino acid sequences appear to be chimeric with amino acid similarities to both, TLR5-stimulating and non-stimulating flagellins. We hypothesised that FlaC might be involved in host immune modulation. For characterising this hypothetical function, coincubation experiments of highly purified FlaC with chicken and human cell lines were performed. FlaC was able to activate different cell types, and preincubation with FlaC reduced the responsiveness of chicken and human macrophages towards bacterial LPS. Additionally, FlaC was shown to directly interact with TLR5 and appeared to be immunogenic in chicken.

Conclusion: We propose that *Campylobacter* spp. have evolved the novel host stimulatory chimeric flagellin-like molecule FlaC in order to specifically modulate host responses, particularly towards other bacterial PRR ligands, and to act predominantly as a homeostatic or tolerogenic signal in the intestinal tract in the presence of the resident microbiota.

(1) Young KT, Davis LM, Dirita VJ. 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat Rev Microbiol. 5(9): 665-679.

(2) Watson RO, Galan JE. 2005. Signal transduction in *Campylobacter jejuni*-induced cytokine production. Cell Microbiol. 7: 655-665.

HUMAN CORONAVIRUS REPLICATION IS CYCLOPHILIN A-DEPENDENT AND INHIBITED BY NOVEL NON-IMMUNOSUPPRESSIVE CYCLOSPORINE A-DERIVATIVES INCLUDING ALISPORIVIR AND NIM811

A. von Brunn[1], J. Carbajo-Lozoya[1], Y. Ma-Lauer[1,2], B. von Brunn[1],P. Mayrhofer[1], R. Hilgenfeld[2], S. Kallies[3], D. Muth[3], M.A. Müller[3], C. Drosten[3], S. Ciesek[4], M. Maleševič[5], G. Fischer[6]

[1]Max Von Pettenkofer Institut, Lmu München, München, Germany, [2]Institute of Biochemistry, University of Lübeck, Lübeck, Germany, [3]Department for Virology, Universität Bonn, Bonn, Germany, [4]Klinik Für Gastroenterologie, Hepatologie Und Endokrinologie, Medizinische Hochschule Hannover, Hannover, Germany, [5]Institue of Biochemistry and Biotechnology, Martin Luther University Halle Wittenberg, Halle, Germany, [6]Max Planck Institute of Biophysical Chemistry Göttingen, Bo Halle, Mpi, Halle (Saale), Germany

Although desperately needed, neither vaccines nor therapeutics are available blocking CoV infection in humans and animals. We reasoned that knowledge of host cell proteins that take part in pivotal functions of the virus-host interplay by interaction with viral proteins and the inhibition of these interactions should define broad-spectrum antiviral cellular targets. This approach should also circumvent the usual fast development of drug resistances observed in the case of viral targets. Screening the SARS-CoV orfeome against human cDNA libraries by unbiased Y2H methods we recently had identified CoV proteins as interaction partners of human cyclophilins (Cyps). Consequently, we showed that Cyclosporin A (CsA) inhibits the replication of human and animal CoVs. Now we demonstrate that novel non-immunosuppressive derivatives of CsA as well as Alisporivir, NIM811 strongly inhibit the growth of human and animal CoVs at low micromolar, non-cytotoxic concentrations in cell culture. We show by qPCR analysis that virus replication is diminished by several orders of magnitude down to background levels. Knockdown of the cellular CypA (CypA/PPIA) in Caco-2 cells prevents replication of HCoV-NL63 respectively, suggesting that CypA is required for virus replication. Collectively, our results uncover CypA as a host target for controlling CoV infection and provide new strategies for urgently needed therapeutic approaches.

SEROLOGICAL EVIDENCE OF INFLUENZA A VIRUSES IN BATS FROM AFRICA

G.S. Freidl[1,2], T. Binger[3], M.A. Müller[3], E. de Bruin[2,1], J. van Beek[1,2], V.M. Corman[3], A. Rasche[3], J.F. Drexler[3], A. Annan[4,5], S. Oppong[4,5], Y. Adu-Sarkodie[4,5], M. Tschapka[6], V.M. Cottontail[6], M. Knörnschild[6], C. Drosten[3], M. Koopmans[1,2]

[1]Viroscience Department, Erasmus Medical Center, Rotterdam, Netherlands, [2]Department of Virology, National Institute for Public Health and the Environment (Rivm), Bilthoven, Netherlands, [3]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [4]Kwame Nkrumah University of Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [5]Virology, Kumasi Collaborative Center for Tropical Medicine, Kumasi, Ghana, [6]Institute of Experimental Ecology, University of Ulm, Ulm, Germany

Bats are known natural hosts for a range of zoonotic viruses such as Marburg, Ebola, Rabies, Nipah, Hendra, as well as for various Corona- and Paramyxoviruses. In 2009/10, researchers discovered RNA of two novel influenza virus subtypes – H17N10 and H18N11 – in Central and South American fruit bats. Although the newly discovered bat influenza virus genome diverged significantly from previously known influenza viruses, evidence suggests that conditions are met to reassort with human influenza viruses in human cells. The identification of bats as possible additional reservoir for influenza A viruses raises questions about the role of this mammalian taxon in influenza A virus ecology and possible public health relevance. As molecular testing can be limited by a short time window in which the virus is detectable, serological testing provides information about past infections and virus spread in populations, after the virus has been cleared.

This study aimed at screening serum from 101 frugivorous bats (*Eidolon helvum*) sampled in 2009/10 in Ghana, for the presence of antibodies against the complete panel of influenza A haemagglutinin (HA) types ranging from H1 to H18 by means of a protein microarray platform. This technique enables simultaneous serological testing against multiple recombinant HA-types in 5µl of serum. Preliminary results indicate serological evidence against avian influenza virus HA-type H9 in 21% of the animals screened, with somewhat cross-reactivity to phylogenetically more closely related HA-types H8 and H12. To our knowledge, this is the first report of serological evidence of influenza A viruses other than H17 and H18 in bats. As avian influenza subtype H9 is associated with human infections, the implications of our findings from a public health context remain to be investigated. Gudrun Freidl and Tabea Binger: These authors contributed equally. Christian Drosten, Marion Koopmans: Shared senior authorship

EPITHELIAL CELL LINES FROM BATS, RODENTS AND INSECTIVORES – A NOVEL TOOL FOR *IN VITRO* INVESTIGATION OF PATHOGEN-HOST INTERACTION

I. Eckerle[1], R. Ulrich[2], A. Rang[3], B. Klempa[3,4], L. Radosa[3,4], M.A. Müller[1], C. Drosten[1]

[1]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [2]Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany, [3]Institute of Virology, Charité, Berlin, Germany, [4]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Despite the great interest created by emerging zoonotic viruses, there is still a lack of *in vitro* models that adequately reflect the exclusive virus-host adaptation of most zoonotic viruses. While the majority of species involved in zoonotic transmission are not available as laboratory animals due to their conservational status or the inability to breed them in captivity, cell lines derived from those species can serve as an acceptable surrogate to study zoonotic viruses in the natural host context.
We have recently established a broad range of reservoir-derived cell lines from bats, providing important insight into immunological and reservoir-host specific mechanisms of zoonotic viruses. However, there has been no focus to selectively culture epithelial cells from zoonotic reservoir hosts so far. The epithelia of the respiratory and renal tract are the predominantly involved cell types in terms of virus entry, replication and shedding. During airborne transmission, it is the first tissue encountered by viral particles and therefore serves as an important barrier of inter-species transmission.
Here, we present an algorithm for establishment and characterization of epithelial cell lines derived from trachea and kidney samples of small mammals. The approach focuses on generation of primary cells derived from samples collected in the field in order to cover a broad range of important reservoir species, including those species that cannot be held in captivity.
Part of this work is funded by the German Research Platform for Zoonoses in the interdisciplinary cross-sectional project "EpiZell". First results of the project include the establishment of epithelial cell lines from important hantavirus reservoir hosts such as *Myodes glareolus*, *Apodemus agrarius* and *Apodemus flavicollis*. Cells were successfully cultured under standardized conditions from both fresh and frozen organ specimens and immortalized for the generation of permanent cell lines. Virus infections studies showed susceptibility and efficient replication of zoonotic viruses, including those that are associated with the respective species in the field.

EXPLORING THE METABOLIC INTERFACE BETWEEN THE GASTROINTESTINAL PATHOGEN CAMPYLOBACTER, ITS HOST AND THE MICROBIOTA

J. Mohr[1], H. Vorwerk[1], C. Huber[2], P. Grüning[3], K. Methling[4], A. von Altrock[5], O. Wensel[1], S. Bhuju[6], J. Kamphues[7], T. Alter[8], A. Flieger[9], K. Schmidt-Hohagen[10], D. Schomburg[10], M. Lalk[4], W. Eisenreich[2], P. Valentin-Weigand[3], D. Hofreuter[1]

[1]Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany, [2]Lehrstuhl Für Biochemie, Center of Isotopologue Profiling, Technische Universität München, Munich, Germany, [3]Institute for Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany, [4]Institute of Biochemistry, Ernst Moritz Arndt University of Greifswald, Greifswald, Germany, [5]Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Services, University of Veterinary Medicine Hannover, Hannover, Germany, [6]Genome Analytics Group, Helmholtz Centre for Infection Research, Braunschweig, Germany, [7]Institute of Animal Nutrition, University of Veterinary Medicine Hannover, Hannover, Germany, [8]Institute of Food Hygiene, Free University of Berlin, Berlin, Germany, [9]Division of Enteropathogenic Bacteria and Legionella, Robert Koch Institute, Wernigerode, Germany, [10]Department of Bioinformatics and Biochemistry, Technical University Braunschweig, Braunschweig, Germany

Campylobacteriosis is the most frequent bacterial enteritis worldwide primarily caused by *Campylobacter jejuni* and to a lesser extent by *Campylobacter coli*. Both food-borne pathogens are closely related and trigger indistinguishable disease manifestations. While *C. jejuni* is most often associated with poultry, it can rarely be found in pigs, the primary reservoir of *C. coli*. Our and other infection studies in pigs suggest that *C. jejuni* and *C. coli* exhibit a different spatial distribution in the porcine gastrointestinal tract of juvenile animals: *C. jejuni* colonizes primarily the jejunum, ileum as well as the caecum and in lower quantities the colon, whereas *C. coli* was recovered form the colon, caecum and ileum but not from the jejunum of pigs. The reason for this tissue tropism is unknown, and we hypothesized that different metabolic properties of the two pathogens could account for the ability of *C. coli* to persist in the large intestine more efficiently than *C. jejuni*. By combining phenotype microarray analysis, comprehensive *in vitro* growth experiments and isotopologue profiling studies, we could identify distinct growth substrate utilization patterns for *C. coli* in comparison to *C. jejuni*. Whole genome sequencing allowed us to elucidate the differences in the catabolic capacities between the two *Campylobacter* species. Furthermore, our metabolome analysis from different gut sections of pigs revealed a clear spatial distribution of nutrients

along the intestinal anterior-posterior axis. Interestingly, *C. coli* but not *C. jejuni* is able to utilize certain nutrients that are abundant fermentation products of the microflora found in the large intestine of pigs. This observation suggested that *C. coli* might benefit more from the metabolic activity of the resident bacteria in the large intestine of pigs than *C. jejuni.* Taken together our study revealed new insights how different physiological properties could influence the distinct tissue tropism of two closely related *Campylobacter* species.

CLONALITY AMONG ESBL-PRODUCING *E. COLI* OF SEQUENCE TYPES (ST) 131, ST410 AND ST167 ISOLATED FROM HUMAN CLINICAL AND AVIAN WILDLIFE SAMPLES FROM THE CITY OF BERLIN, GERMANY

M. Wöhrmann[1], K. Schaufler[2], K. Müller[3], T. Semmler[2], R. Leistner[4], A. Kola[4], L.H. Wieler[2], S. Guenther[2]

[1]Freie Universität Berlin, Centre for Infection Medicine, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [2]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [3]Freie Universität Berlin, Clinic for Small Animals, Berlin, Germany, [4]Charité Universitätsmedizin Berlin, Institut Für Hygiene Und Umweltmedizin, Berlin, Germany

Over the last years, the occurrence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* in wildlife has been reported from all over the world. To understand spatial small scale transmission scenarios and to evaluate an indicator function of wild birds for an environmental pollution with multi-resistant bacteria we screened avian wildlife from the Berlin area (Germany) and compared ESBL-producing strains to human and veterinary clinical isolates from the same area at the same time point.
Therefore, a total of 320 wild birds were screened for ESBL-producing *E. coli* during entrance examination in a small animal clinic using cloacal swabs and selective plating (4 µg/ml cefotaxime). Isolate characterisation included ESBL-confirmatory testing, MIC- testing, PCR screening for ESBL-resistance determinants like blaCTX-M, blaTEM and blaSHV as well as non-beta-lactam resistance genes including tetA-C, sul1-3, and strA/B. The phylogenetic background was determined via Multilocus sequence typing (MLST) and STRUCTURE analysis. All ESBL-producers independent from their origin were additionally screened for clonal relatedness via pulsed-field gel electrophoresis (PFGE). For comparative reasons, 40 human clinical ESBL-producing isolates from a large University hospital were analysed using the same methods.
Results: Overall, eight percent of the sampled birds carried ESBL- or AmpC-producing Enterobacteria. The ESBL-resistance in both human and animal samples was always encoded on the genes blaCTX-M-1 or blaCTX-M-15. MLST analysis found that almost all avian ESBL-producers belonged to typical ESBL-sequence types like ST131, ST648, ST617, ST224 or ST167. Comparison via PFGE detected clones of ESBL-producing isolates from ST131, ST410 and ST167 originating from wild birds, humans and companion animals.
Conclusions: Wild birds carry substantial numbers of ESBL-producing *E. coli* resembling clinical relevant strains from human and veterinary medicine, pointing towards an environmental pollution by multi-resistant *E. coli* clones from medical facilities as well as a certain environmental fitness of certain ESBL-producing lineages.

VARIOUS WAYS OF SHEDDING OF BORNA DISEASE VIRUS IN LIVING BICOLORED WHITE-TOOTHED SHREWS

D. Nobach[1], M. Bourg[1], S. Herzog[2], H. Lange-Herbst[2], J.A. Encarnação[3], M. Eickmann[4], C. Herden[1]

[1]Institute of Veterinary Pathology, Justus Liebig University, Giessen, Germany, [2]Institute of Virology, Justus Liebig University, Giessen, Germany, [3]Department of Animal Ecology Systematics, Justus Liebig University, Giessen, Germany, [4]Institute of Virology, Philipps University, Marburg, Germany

Borna Disease Virus (BDV) belongs to the order *Mononegavirales* which comprises many RNA viruses belonging to the families *Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae* with natural reservoirs and zoonotic potential. The neurotropic BDV affects lethally the nervous system in end hosts such as horses and sheep. In the recently detected natural viral reservoir, the bicolored white-toothed shrew (*Crocidura leucodon*), BDV-infection seems to run an inapparent course. To further characterize viral maintenance in reservoir species, bicolored white-toothed shrews were caught alive. In three living bicolored white-toothed shrews BDV was detected enabling the investigation of natural infection with BDV in this reservoir.

Despite harbouring infectious virus the animals were clinically inconspicuous. BDV-RNA was detected by RT-PCR in various excretions and secretions as saliva, urine, sebum, lacrimal fluid and faeces. Furthermore, viral RNA was present in the ground substrate from their lairs. Infectious virus was isolated from saliva, sebum and urine on rabbit embryonic brain cells. Virus shedding corresponds well to the morphological demonstration of viral antigen and RNA in the respective organ systems visualized by immunohistochemistry and in situ hybridization.

Taken together, there are various ways of shedding BDV in the bicolored white-toothed shrew which enables successful viral maintenance in the reservoir population and even fatal transmission to susceptible end hosts such as horses and sheep.

SURVEILLANCE OF ERADICATION OF MRSA AND ESBL-E ON A MODEL PIG FARM

R. Schmithausen[1], S. Kellner[2], S. Schulze-Geisthoevel[2], S. Hack[3], G. Bierbaum[1], A. Hoerauf[1], M. Exner[3], B. Petersen[2], I. Bekeredjian-Ding[1]

[1]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital of Bonn, Bonn, Germany, [2]Department of Preventive Health Management, Institute of Animal Science, University Hospital of Bonn, Bonn, Germany, [3]Institute of Hygiene and Public Health, University Hospital of Bonn, Bonn, Germany

Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-Spectrum-ß-Lactamase bearing Enterobacteriaceae (ESBL) were tested as indicator bacteria of recontamination in pigs, dust, air and water on a pig-producing farm before and after total decontamination of the stable. The question of the study was: Is it possible to achieve a permanent decontamination of a pig farm by a complete exchange of pigs and a total decontamination of the former stable and the construction of an additional new stable?

The study was planned as a two-step approach. In the first step samples of pigs, humans, dust, air and water were obtained in the original stable (before decontamination). Then all sampled pigs were slaughtered, followed by a professional decontamination (DESTEC GmbH) of the stable and construction of a new nearly identical stable. In the second step, incoming pigs (previously identified as MRSA and ESBL negative) as well as humans, dust, air and water were immediately sampled in May with monthly repeats for 3 months.

In total 1242 samples were obtained (426 pig, 12 human, 368 dust, 138 air, 298 water samples). MRSA were detected in 40.3% of the pig samples before compared to 42.6%/41%/20% (May/June/July) after decontamination. MRSA was isolated in 25% of the dust samples before decontamination and in 5.6%/21.8%/14.5% (3 months repeats) afterwards. Regarding the air samples all were MRSA positive before decontamination unlike 33.3% (May) (38.5% in June/July) positive samples after decontamination. The contamination rates with MRSA of the water (samples taken in every compartment) were documented with 18.9% before and with 5.4% (May) respectively 2.6% (June/July) after decontamination.

ESBL-producing bacteria were only detected in anal swabs of pigs. Whereas 37.1% of pigs were ESBL positive before decontamination but no pig was ESBL positive after decontamination. Overall, differences of the contamination rates between the decontaminated and the new stable were not significant.

A resistance-free environment could not be created and a reduced prevalence of MRSA in pigs by professional decontamination and new-construction of the stables was not achieved. However, the MRSA prevalence in stable environment (dust, air, water) could be reduced by strict disinfection measures and hygiene management.
The contamination level of ESBL in pigs could be reduced initially but was not stable over time.The transmission pathways for new- or possibly re-contamination need to be identified.

MOLLUSCAN VIBRIO SPECIES IN CANADA'S ESTUARIES: A LONGITUDINAL STUDY OF TREND AND DYNAMICS IN TEMPERATE WATERS

S.K. Banerjee, J.M. Farber

Bureau of Microbial Hazards, Food Directorate, Health Canada, Ottawa, Canada

Introduction: The occurrence and survival of particular *Vibrio* species are linked to the temperature, salinity and nutrient status of the coastal waters, globally. We pursued the trend and dynamics of molluscan *Vibrio* community detected in the estuaries of Canada, influenced by the temperate waters of the Atlantic or the Pacific Ocean.

Methodology: Bivalve molluscs (clams, mussels, oysters) were sampled from the coastal waters of Canada, for the presence of clinically significant *Vibrio* species, such as *V. parahaemolyticus* (Vp), *V. vulnificus* (Vv) and *V. cholerae* (Vc), from May to October of each year from 2002 to 2013. Biochemical and molecular assays, including polymerase chain reaction (PCR), were used to identify and characterize the isolates. Other *Vibrio* spp., such as *V. alginolyticus* (Va) and *V. fluvialis* (Vf), were included in the study from 2007.

Results: Out of 531 molluscan samples tested during the 12-year period, a trend was observed in the composition of the vibrio community. During the first four years, Vp was detected in 45% of the 126 samples tested. From 2007 to 2013, the spectrum of *Vibrio* population appeared broader with the detection of Va (93%), Vp (49%), Vv (16%), Vf (10%), Vc (2%) and unidentified *Vibrio*spp. of marine origin (10%). Overall, 13% of the samples tested positive for pathogenic Vp by PCR, during the 12-year period with an increasing frequency in Atlantic samples. If the study period was divided into three 4-year segments, the frequency of samples with pathogenic Vp changed from 14% to 7% to 9% in the West, and 2% to 15% to 28% in the East coast molluscs, respectively. Interestingly, one isolate of Va isolated in 2007 from the West coast, tested positive for a pathogenic marker.

Conclusions: A significant outcome of this study was the isolation and identification of potentially pathogenic strains of Vp with a higher prevalence than in previous years. Dynamics of the *Vibrio*species and the trend observed in diversity may be products of environmental pressures, coinciding with global warming. Science-based knowledge is documented to provide insight into the future of food safety and regulatory intervention.

AN OBSERVATIONAL STUDY OF AN OUTBREAK OF MERS-CORONAVIRUS IN JEDDAH, KSA, IN 2014

D. Muth[1], V. Corman[1], R. Hussain[2], H. Madani[2], A. Zumla[3], A. Al Shangiti[4], Z. Memish[4], C. Drosten[1]

[1]University of Bonn Medical Centre, Institute of Virology, Bonn, Germany, [2]Jeddah Regional Laboratory, Virology Unit, Jeddah, Saudi Arabia, [3]University College London, Division of Infection and Immunity, London, United Kingdom, [4]Ministry of Health Saudi Arabia, Departments of Preventive Medicine and Infection Prevention and Control, Riyadh, Saudi Arabia

In April 2014 the number of notified MERS-Coronavirus infections in Jeddah, KSA, experienced a sudden and dramatic rise. This caused disconcertment about a possible change in epidemiological parameters of the infection. Explanations might be an increase of zoonotic transmissions, increase of person-to-person spread, increased patient testing, laboratory artifacts or even changes is virus properties. In an ad-hoc collaborative study we investigated essential features of the outbreak.

Viruses associated with the outbreak were monophyletic as compared to viruses from an outbreak taking place simultaneously in Riyadh. Appropriate calculations were done after sequencing the 3' genomic region including and downstream of the spike gene. Additionally, virus isolates were obtained from both outbreaks and compared to the "gold standard" MERS-CoV strain EMC/2012, the first human isolate. Cell culture experiments revealed similar virus growth, same sensitivity of viruses to interferon treatment and equal neutralization of all viruses by anti-MERS antibodies. No change in virus shedding was observed over the course of the outbreak in Jeddah. Despite the massive increase of performed diagnostic tests, no carry-over contamination was observed during an assessment of laboratory proficiency. Interestingly, patient samples with very low Ct values appeared very often as compared to the outbreak in Riyadh suggesting a low-level virus replication.

Our data dismiss the possibility of modified virus functions or laboratory-related issues due to increased diagnostic requests during the Jeddah outbreak. Increased alertness and altered MERS-case definition is the most likely explanation for the extent of the described outbreak. While close investigations of MERS and MERS contacts is highly interesting from a scientific point of view, we should be careful not to overwhelm the healthcare system. Testing should be accompanied by straight guidelines regarding home quarantine and re-testing minimizing public concerns.

TRICHINELLOSIS – A NEGLECTED ZOONOSIS IN GERMANY?

K. Nöckler, S. Reckinger, A. Mayer-Scholl

Department for Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

Trichinellosis is an important foodborne, zoonotic diseases and is caused by nematodes of the genus *Trichinella*. Human infections occur through consumption of raw or inadequately processed meat or meat products. Therefore, susceptible animals intended for food must undergo meat inspection and positive findings have to be verified for the *Trichinella* species.

According to German data from meat inspection in domestic animals, *Trichinella* infection is negligible in domestic pigs and horses where the prevalence in 2012 was less than 0.000002% and 0%, respectively. Conversely, *Trichinella* is autochthonous in the sylvatic cycle in Germany as demonstrated in a wildlife monitoring in 2011 in raccoon dogs and foxes where the average prevalence was 0.3% and 2.6%, respectively. The average prevalence in wild boars is 0.003%. Between 2002 and 2013, more than 160 isolates were obtained from wild boar, foxes, raccoon dogs, swine, wolves and badgers, whereby *T. spiralis* was the predominant species followed by *T. pseudospiralis*, *T. britovi* and *T. nativa*.

From 2001-2013, a total of 79 human trichinellosis cases (average 6.1 cases, median 3 cases per year) was reported for Germany. Most cases were related to "imported diseases" from regions, where trichinellosis is still a public health threat. However, autochthonous human trichinellosis outbreaks occurred in 2006 and 2013 due to consumption of pork and wild boar meat, respectively. These outbreak clusters are located in the North-Western parts of Germany where most of *Trichinella* isolates were detected not only in wildlife but also in domestic pigs from private holdings with outdoor access.

Data from human trichinellosis cases and *Trichinella* findings from meat inspection and wildlife monitoring demonstrate that a residual risk for exposure may exist for consumers. Therefore *Trichinella* meat inspection is compulsory for food animals with a relevant risk of infection with this parasite (e.g. wild boars, pigs kept outdoors). Consumers should be informed about the risk of eating raw or undercooked meat/products and physicians should be aware of diagnostic and therapeutic features especially in regions where trichinellosis is a rare disease.

EFFICACY TESTING OF INACTIVATION METHODS FOR FILOVIRUSES

E. Haddock[1], F. Feldmann[2], H. Feldmann[1]

[1]Laboratory of Virology, Dir/niaid/nih, Hamilton, Mt, United States, [2]Rocky Mountaint Veterinary Branch, Dir/niaid/nih, Hamilton, Mt, United States

Reliable inactivation of samples prior to removal from High Containment Laboratories (BSL3/4) is crucial for safe operation and mandatory under the select agent program. Specimens are commonly inactivated using long established, widely used approaches that are based on past experience and not well documented safety testing. In order to re-evaluate inactivation efficacy of commonly used procedures, we have compared *in vitro* and *in vivo* approaches using Zaire ebolavirus as a representative viral pathogen. For *in vitro* testing we used wild-type Zaire ebolavirus expressing enhanced green fluorescence protein, which allows for cytopathic effect and fluorescence as simple rapid read out. For *in vivo* testing we utilized mouse-adapted Zaire ebolavirus infection of BALB/c mice, an extremely sensitive model with a LD50 of 0.01 focus forming units. Different biological specimens (i.e. virus supernatant and/or infected cells) were inactivated by gamma-irradiation, guanidinium isothiocyanate-based buffers, Trizol, sodium dodecyl sulfate, paraformaldehyde, glutaraldehyde, formalin and heat; samples were dialyzed if necessary prior to safety testing. In conclusion, we have established parameters leading to proper inactivation and found that *in vitro* safety testing is as reliable as *in vivo* testing. The established protocols are easy to perform and sufficiently reliable. Our findings support the implementation of standard operating protocols that allow for removal of samples from BSL3/4 without prior safety testing of individual samples if those approved protocols were applied for inactivation.
The study was supported by the Intramural Research Program, NIAID, NIH.

ESBL-PRODUCING *E. COLI* FROM ANIMAL AND HUMAN SOURCES – WHAT DO THEY SHARE?

H. Sharp[1], L. Valentin[1], K. Hille[2], U. Seibt[3], J. Fischer[1], Y. Pfeifer[4], G. Brenner Michael[5], S. Nickel[6], J. Schmiedel[7], L. Falgenhauer[7], A. Friese[8], R. Bauerfeind[9], U. Rösler[8], C. Imirzalioglu[7], T. Chakraborty[7], R. Helmuth[1], G. Valenza[6], G. Werner[4], S. Schwarz[5], B. Guerra-Roman[1], B. Appel[1], L. Kreienbrock[2], A. Käsbohrer[1]

[1]Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany, [2]Department of Biometry, Epidemiology and Information Processing, Who Centre for Research and Training in Veterinary Public Health, University of Veterinary Medicine Hannover, Hannover, Germany, [3]Institute of Pharmacology, Pharmacy and Toxicology, University of Leipzig, Leipzig, Germany, [4]Nosocomial Pathogens and Antibiotic Resistances, Robert Koch Institute, Wernigerode, Germany, [5]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt Mariensee, Germany, [6]Bavarian Health and Food Safety Authority, Lgl, Erlangen, Germany, [7]Institute for Medical Microbiology, Justus Liebig University, Giessen, Germany, [8]Institute for Animal Hygiene and Environmental Health, Free University of Berlin, Berlin, Germany, [9]Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University, Giessen, Germany

Escherichia (*E.*) *coli* producing extended-spectrum beta-lactamases (ESBLs) are an increasing problem for public health. The success of ESBLs may be due to both, spread of ESBL-producing bacterial clones and transfer of ESBL gene-carrying plasmids. This makes it difficult to identify exposure routes and sources for ESBL-producing bacteria. The objectives of this study were to statistically analyze and compare the genotypic and phenotypic properties of *E. coli* isolates from different animal and human sources.

ESBL-producing *E. coli* from two longitudinal and four cross-sectional studies in broiler, swine and cattle farms, one case-control and one cross-sectional study in humans and diagnostic isolates from humans and animals were used. All studies were part of the German RESET consortium and all laboratories followed harmonized methodologies for antimicrobial susceptibility testing, confirmation of the ESBL phenotype, specific PCR assays for the detection of bla_TEM, bla_CTX-M, and bla_SHV genes and sequence analysis of the complete ESBL gene as well as a multiplex PCR for the detection of the four major phylogenetic groups of *E. coli*.

For analyzing the distribution of ESBL-producing *E. coli* subtypes among the different populations, the subtype of each isolate was defined by a combination of the genotype of the ESBL genes found, its phylogenetic group and its susceptibility against certain antimicrobials. Most ESBL genes were found in both, human and non-human populations although

quantitative differences for distinct ESBL types were detectable. When *E. coli* subtype definition included phylogenetic grouping and antimicrobial susceptibility data, the proportion of common clusters was markedly reduced. Nevertheless, relevant proportions of same subtypes was detected in isolates from the human and livestock and companion animal populations included in this study, suggesting some interactions between these populations. Our current approach provides good insight into common and population-specific clusters, which can be used as a basis for the selection of ESBL-producing isolates for further detailed characterizations, e.g. by whole genome sequencing.

MRSA INFECTIONS IN COMPANION ANIMALS: CHARACTERIZATION OF RISK FACTORS

S. Vincze[1], A.G. Brandenburg[2], W. Espelage[3], I. Stamm[2], L.H. Wieler[1], P.A. Kopp[2], A. Lübke-Becker[1], B. Walther[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany, [2]Vet Med Labor Gmb H, Division of Idexx Laboratories, Ludwigsburg, Germany, [3]Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany

Infections with methicillin-resistant S. aureus (MRSA) are a burden in both, human and veterinary medicine. Especially companion animals such as dogs, cats and horses suffered increasingly from MRSA infections in recent years. Since MRSA are frequently multidrug-resistant, these infections lead to serious antibiotic treatment difficulties. In order to limit MRSA infections in future, targets for effective intervention have to be identified. Therefore, we conducted a case-control study including 194 companion animals with MRSA (n=100) or MSSA (n=94) infections. The isolates were obtained from 155 different veterinary practices and originated from various infection sites.
All S. aureus were initially isolated from routine diagnostic and identified by use of the Vitek2 system (bioMérieux). Genotypic confirmation was carried out by detection of the nuc gene and the methicillin-resistance encoding mecA gene. Veterinarians who received an MRSA or MSSA positive result from the diagnostic lab were invited to take part in this study by replying to a questionnaire. Information for each case (MRSA) and control (MSSA) included age, sex, breed and clinical history. Further, veterinarians provided information on staff and size of their veterinary practice. Putative risk factors were analyzed using univariable logistic regression (likelihood ratio test) including all parameters from patients and veterinary settings. Risk factors with $p<0.2$ were integrated in the final multivariable model using manual stepwise backward elimination (multinomial logistic regression).
Patients with antibiotic treatment prior sampling and surgical site infections were more likely to suffer from MRSA infections in comparison to MSSA infections. Further, animals that had been treated in practices or clinics with more than ten employees were at a higher risk to be infected with MRSA.
The identification of risk factors for MRSA infections that are clearly associated with the veterinary setting like clinic size or surgical site infection highlights the importance of MRSA as nosocomial pathogen in companion animals.

PATHOGENIC NEMATODES FOUND IN FISH AND FISHERY PRODUCTS MADE OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) FROM MARKETS IN POLAND

M. Rozycki, E. Bilska-Zajac, E. Chmurzynska, T. Cencek, M. Kochanowski

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pulawy, Poland

The aim of the study was to determine the frequency and intensity of roundworm infections in highly valued fish and fishery product (mainly fillets) made from the muscle tissue of pink salmon present on the market and species identification of parasites detected. Pink salmon were caught by a commercial seiner in FAO 67 region. For the explanation of the area 67, are marine waters of the Northeast Pacific from a the mainland coast of Russia in the Western Bering Sea at 175°00'W, along the coast to Mys Dazhneva, hence through the Bering Strait to Cape Prince of Wales and in southeast direction to the mainland coast of Alaska to the 130°00'W longitude and to 40°00'N latitude. For the study over 150 samples were taken. Samples were collected from 2009 to 2012. All samples were taken at frozen stage. The average weight of the sample was 250g. Boneless, skinless fillets were placed in polyethylene bags and deiced overnight. Priori to examination fillets were gently grind by hand. Parasites were isolated by artificial digestion method. Digestion method (ZP/PB-45) was validated and accredited by Polish Centre for Accreditation (PCA). Found nematodes were identified on the base of morphological characteristics according to Grabda key identification of marine parasite entities guide. Species confirmation was performed by PCR/RFLP (EURLP developed and validated method). Parasites were found in 93 (62 %) samples. Larvae were identified on the base of morphological characteristic as *Anisakis spp.*. and *Pseudoterranova decipiens*. No other parasites were found in the examined samples. Species identification were confirmed by PCR/RFLP method. Over 96 % of nematodes were identified as *Anisakis simplex sensu stricto* and 4 % as *Pseudoterranova decipiens*. Obtained results confirm the prevalence of human pathogenic and allergenic nematodes in highly valued fish products. Presented work is the part of studies on set of methods for detection of nematodes in fish and fish products of particular importance due to their pathogenicity and allergenicity for humans.

MOLECULAR DETERMINANTS OF VIRULENCE OF EMERGING TICK-BORNE PHLEBOVIRUSES: FROM PHLYLOGENETIC TREES TO THE MOLECULAR BASIS OF PATHOGENESIS

K. Matsuno, C. Matysiak, C. Weisend, A. Groseth, H. Ebihara

Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, United States

A novel tick-borne phlebovirus (TBPV), Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), has emerged in Eastern Asia where it causes a severe and often fatal febrile illness with thrombocytopenia in humans. Notably, a genetically related phlebovirus, Heartland virus (HRTV), has been identified in the United States from patients exhibiting a febrile, sometimes fatal, illness with thrombocytopenia. The emergence of these two related viruses on two different continents strongly suggests that other unrecognized and potentially pathogenic TBPVs are distributed worldwide. Indeed, two novel SFTSV-like viruses, Hunter Island virus (HUIV) and Malsoor virus (MALV), were discovered in ticks and bats in Australia and India, respectively. By conducting genome sequencing of taxonomically unassigned bunya-like viruses isolated from ticks, we identified Bhanja virus (BHAV), which is distributed throughout Eurasia and Africa where it causes a febrile illness with central nervous system involvement in humans, as a close relative of SFTSV within the genus *Phlebovirus*. This phylogenetic analysis has allowed us to divide TBPVs into three distinct groups based on their genetic/serological similarities and virulence: (1) the SFTSV/HRTV group associated with hemorrhagic fever-like illness; (2) the BHAV group associated with sporadic febrile disease; and (3) the Uukuniemi virus (UUKV) group, which is not associated with illness in humans. To identify molecular determinants of SFTSV/HRTV pathogenicity in human, we focused on the NSs protein, which is known as a phleboviral interferon (IFN) antagonist. We expressed the NSs proteins of various TBPVs and compared their abilities to inhibit IFN-β induction and signaling pathways. Interestingly, we found that NSs of SFTSV/HRTV and BHAV group viruses exhibited a strong ability to inhibit IFN-β promoter activity; whereas, the NSs of the UUKV group viruses and, surprisingly, HUIV and MALV, were unable to inhibit IFN-β activity. In addition, only the SFTSV/HRTV group NSs showed a strong ability to inhibit type I IFN signaling via the Jak/STAT pathway. Overall, this work demonstrates a strong correlation between NSs function and TBPV pathogenicity in humans, which suggests that the ability of NSs to antagonize IFN can be considered a molecular signature of virulence and used to predict the future emergence of TBPVs.

AN INFECTIOUS BAT CHIMERIC INFLUENZA VIRUS HARBORING THE ENTRY MACHINERY OF A CONVENTIONAL INFLUENZA A VIRUS

M. Juozapaitis[1], É. Aguiar Moreira[1], I. Mena[2], S. Giese[1], D. Riegger[1], A. Pohlmann[3], D. Höper[3], G. Zimmer[4], M. Beer[3], A. García-Sastre[2], M. Schwemmle[1]

[1]Department for Microbiology and Hygiene, Institute for Virology, University Clinic Freiburg, Freiburg, Germany, [2]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, United States, [3]Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald, Germany, [4]Institute of Virology and Immunology, Ivi, Mittelhäusern, Switzerland

In 2012 the complete genomic sequence of a new and potentially harmful influenza A-like virus from bats (H17N10) was identified. However, infectious influenza virus could be neither isolated from infected bats nor reconstituted, impeding further characterization of this virus. Here we show the generation of an infectious chimeric virus containing six out of the eight bat virus genes with the remaining two genes encoding the HA and NA proteins of a prototypic influenza A virus. This engineered virus replicated well in a broad range of mammalian cell cultures, human primary airway epithelial cells and mice, but poorly in avian cells and chicken embryos without further adaptation. Importantly, the bat chimeric virus is unable to reassort with other influenza A viruses. Although our data does not exclude the possibility of zoonotic transmission of bat influenza viruses into the human population, they indicate that multiple barriers exist that makes this a very unlikely event.

CHARACTERIZATION OF GUAROA VIRUS GENETIC DIVERSITY, EVOLUTION AND SPREAD

A. Groseth[1], K.K. Wollenberg[2], V. Mampilli[1], T. Shupert[1], C. Matysiak[1],C. Weisend[1], T.J. Kochel[3,4], R.B. Tesh[5], H. Ebihara[1]

[1]Molecular Virology and Host Pathogen Interaction Unit, Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, United States, [2]Office of Cyberinfrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, United States, [3]Viral and Rickettsial Diseases Department, Naval Medical Research Center, Silver Spring, United States, [4]Virology Department, Naval Medical Research Unit 6, Lima, Peru, [5]Department of Pathology and Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, United States

Guaroa virus (GROV) is a frequent but understudied cause of febrile illness throughout South America where it is transmitted by mosquitoes, as part of a cycle that may also include avian and/or rodent hosts. Despite its public health importance, there has been little data available for assessing the genetic diversity among GROV isolates or modelling virus evolution and spread. To address this, complete sequencing of 12 geographically and temporally diverse isolates of GROV was undertaken. Analysis of sequence divergence combined with phylogenetic analysis showed that, with the exception of an early Brazilian isolate (strain BeH22063) for which the only pre-existing full-length sequence data was available, all other isolates were closely related. Based on these data, we have developed pan-GROV primer sets that detect the entire known range of GROV genetic diversity, unlike those based on the BeH22063 strain, which were unreliable for detection of other isolates. In addition, the close molecular and geographical relationship of GROV to the Wyeomyia group viruses (WYOV) allowed us to model virus spread following the introduction of their common ancestor into the Central/South American region. These analyses suggest that the spread of GROV into Peru and Bolivia, where they currently pose a significant problem, is most likely a recent event and that careful monitoring for further expansion of the endemic region is warranted. Further, we have conducted additional evolutionary and ancestral sequence reconstruction analyses combined with *in vitro* experiments, to examine the loss of the major interferon (IFN) antagonist, NSs, in the WYOV group and its impact on virus biology. These studies suggest that NSs was specifically lost in the WYOV lineage and provide experimental evidence that the loss of NSs in WYOVs results in a functional impairment of IFN antagonism, which in turn impairs growth in human macrophages. These studies provide a possible basis for

the apparent attenuation of the WYOVs compared to other closely related viruses, including Guaroa virus, and provides an approach (i.e. combining bioinformatics with the characterization of NSs function) that can be applied to predict the threat to human and animal health posed by the emergence of novel bunyaviruses.

EFFECTS OF CATHELICIDIN ANTIMICROBIAL PEPTIDES AGAINST LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

S. Blodkamp[1], K. Kadlec[2], H.Y. Naim[1], S. Schwarz[2], M. von Köckritz-Blickwede[1]

[1]Department of Physiological Chemistry, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [2]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt Mariensee, Germany

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a colonizer but also an important zoonotic pathogen which is readily exchanged between different animal species and humans. Its - in part - expanded antimicrobial resistance presents a challenge in the control of infections caused by LA-MRSA. One way to overcome this problem is to boost the host immune system against the infection. Possible targets are the endogenous antimicrobial peptides (AMPs) of the host cells. AMPs, such as the cathelicidins, are an essential part of the innate immune system, as they act as signal molecules but also kill pathogens directly.

The aim of this study was to characterize the antimicrobial activities of five different cathelicidins derived from different animal species (LL-37, CRAMP, CAP18, BMAP-27 and BMAP-28) against livestock-associated methicillin-resistant *S. aureus* (LA-MRSA).

For this purpose the minimal inhibitory concentrations (MICs) of 153 field isolates were determined. Moreover, the impact of 14 antimicrobial resistance genes, which specify different resistance mechanisms, on the MICs of cathelicidins was investigated. Therefore, the plasmids carrying different known antimicrobial resistance genes were transferred into S. aureus NewmanΔdlt, a prototype strain that is relatively susceptible for the tested AMPs, to see if the MICs increase.

The results demonstrated that the lowest MIC values were obtained for the bovine cathelicidins, BMAP-27 and BMAP-28 (4-16 µg/ml and 2-16 µg/ml, respectively). The human and mouse cathelicidins, LL-37 and CRAMP, showed the highest MICs (both ≥ 128 µg/ml). These differences of the cathelicidin activities correlate with their hydrophobicity. Interestingly, an effect of antimicrobial resistance genes on the MICs could not be detected.

Since bovine cathelicidins, as revealed in this study, exhibit lower MICs against LA-MRSA compared to cathelicidins of other species, they might be a promising target for pharmacological boosting, especially since none of the tested antimicrobial resistance genes altered the MIC values. Further experiments that clarify the molecular and biochemical basis of the interaction of cathelicidins with bacteria are currently performed.

POSTER PRESENTATIONS

Board No: 1 *Epidemiology, Modeling and Prediction*

ZEBRA-BORNE EQUINE HERPESVIRUS TYPE 1 (EHV-1) INFECTION IN NON-AFRICAN CAPTIVE MAMMALS

A.M. Abdelgawad[1], W. Azab[2], A. Damiani[2], K. Baumgartner[3], H. Will[3], N. Osterrieder[2], A.D. Greenwood[1]

[1]Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, [2]Department of Veterinary Medicine, Institut Für Virologie, Berlin, Germany, [3]Department of Veterinary Medicine, Zoo Nuremberg, Nuremberg, Germany

Equine herpesvirus type 1 (EHV-1) zebra strain was detected in a male polar bear suffering from seizures in the Zoological Garden Nürnberg, Germany. Subsequently, an Indian rhinoceros (Purana) aborted in the late stage of pregnancy suffered nervous disorder resulting in her death. Encephalitis was suspected and viral DNA was detected by regular PCR and quantitative PCR in brain, lung, and spleen tissues. Furthermore, the viral IR6 protein was detected in several tissues, most strongly in lung. Phylogenetic analyses of sequence of gB, IR6, UL45, UL49.5, and DNA polymerase isolated from Purana's tissues were aligned with reference sequences for the same regions of EHV-1, EHV- 9, and EHV-4 confirmed that the virus was nearly identical to a recently described EHV-1 strain that resulted in both non-fatal and fatal encephalitis in polar bears. This represents transmission of EHV-1 to a species that is not naturally sympatric with the natural host of the virus and broadens the host range to Asian non-equid perissodactyls. Importantly, like polar bears, the Indian rhinoceros would never come in contact with zebras in the wild. Thus, the mortality thus far in species that are not con-specifics of zebras may suggest exposure to EHV may yield particularly severe outcomes for non-African mammals. Mixing of geographically dispersed mammals only occurs in zoological collections and circuses which may provide pathogens such as EHVs novel opportunities to disseminate to new hosts. The data suggest that EHV-1 and its close relatives are now prevalent and can infect different animal species with devastating and often fatal outcomes. We contend that EHV-1 should be considered an emerging infectious agent in captive animal populations.

Board No: 2 *Epidemiology, Modeling and Prediction*

VIRAL ZOONOTIC DISEASES IN KAZAKHSTAN

K. Abdiyeva[1], A. Dmitrovsky[1], Z. Shapieva[2], R. Yegemberdieva[1], L. Yeraliyeva[1], S. Frey[3], J. Zinner[3], S. Essbauer[3]

[1]Department of Infectious Diseases, Kazakh National Medical University, Almaty, Kazakhstan, [2]Department of Parasitology, Scientific and Practical Center of Sanitary and Epidemiological Expertise and Monitoring, Almaty, Jordan, [3]Department of Virology and Rickettsiology, Bundeswehr Institute of Microbiology, Munich, Germany

Kazakhstan has a unique geographical location being adjacent to known endemic foci of zoonotic diseases e.g. in Russia and China. Further this country has a wide range of climatic and vegetation zones, some of these having highly favorable conditions for different vectors of zoonotic diseases. Unfortunately, the most popular touristic places of Kazakhstan are endemic regions e.g. for Crimean-Congo Hemorrhagic Fever (CCHF, south Kazakhstan) or tick-borne encephalitis (TBE, Almaty and East Kazakhstan regions). Further formation of new endemic zone has been suspected for hantavirus infections in West Kazakhstan, or TBE in North Kazakhstan in close neighborhood to known Russian TBE foci. Further, in Kazakhstan nosocomial infections with CCHF-virus are often registered. Despite some knowledge on endemic zones of CCHF, TBE and hantavirus infections, the aetiological agents have not been studied in detail. Further there might exist other viruses of the e.g. bunya-, flavi- and picornavirus family inducing severe fever of unknown origin, hemorrhagic fevers or encephalitis. Examples are, Usunagach, Karshi, and Sirdariyavirus. We initiated a project to investigate the seroprevalence of the above mentioned agents among hospitalized patients with fever of unknown origin. Further the prevalence of the agents is investigated in different vectors and reservoirs in order to get more knowledge on the viruses and genetic data of the responsible strains. This work will also support to improve the system of laboratory diagnostics and surveillance of these highly pathogenic agents in Kazakhstan. This project is funded by the German Federal Foreign Office in the framework of the German Partnership Program for Excellence in Biological and Health Security.

Board No: 3 *Epidemiology, Modeling and Prediction*

MOLECULAR DETECTION OF *THEILERIA* AND *BABESIA* SPECIES IN TICKS AND FIRST MOLECULAR EVIDENCE FOR *BABESIA MICROTI* IN TURKEY

M.F. Aydin[1], M. Aktas[2], N. Dumanli[2]

[1]Research Laboratory, Higher Health School, University of Karamanoğlu Mehmetbey, Karaman, Turkey, [2]Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, Elazig, Turkey

Keywords: Babesia, RLB, *Theileria*, tick.

A molecular survey was undertaken in Black Sea region of Turkey to determine the presence of *Theileria* and *Babesia* species of medical and veterinary importance. The ticks were removed from sheep and goats, pooled according to species and locations, and analyzed by PCR-based reverse line blot (RLB) and sequencing. A total of 2241 ixodid ticks belonging to 5 genus and 12 species were collected, and divided into 310 pools. Infection rates were calculated as the maximum likelihood estimation (MLE) with 95% confidence intervals (CI). Of the 310 pools tested, 46 (14.83%) were found to be infected with *Theileria* or *Babesia* species, and the overall MLE of the infection rate was calculated as 2.27% (CI 1.67-2.99). The MLE of the infection rates were calculated as 0.691% (CI 0.171-1.78) in *H. parva*, 1.47% (CI 0.081-6.37) *R. sanguineus*, 1.84% (CI 0.101-7.87) *I. ricinus*, 2.86% (CI 1.68-4.48) *R. turanicus*, 5.57% (CI 0.941-16.3) *H. marginatum* and 6.2% (CI 4.02-9.02) *R. bursa*. Pathogens identified in ticks included *Theileria ovis, Babesia ovis, Babesia bigemina and Babesia microti.* Most tick pools were infected with a single pathogen. However, five pools displayed mixed infections with *T. ovis* and *B. ovis*. This study provides the first molecular evidence for the presence of *B. microti* in ticks in Turkey.

Board No: 4 *Epidemiology, Modeling and Prediction*

PHYLOGENETIC CHARACTERIZATION, PHYLOGEOGRAPHIC DYNAMICS AND GENOMIC FEATURES OF DENGUE VIRUS SEROTYPE 4 STRAINS FROM RIO DE JANEIRO, BRAZIL

R. de Mendonça Campos[1], D. Cadar[2], G. Louzada[1], M. Meneses[1,3], C. Fernandes[3], C. Veiga[3], L. Souza[3], J. Schmidt-Chanasit[2], D. Ferreira[1]

[1]Department of Virology, Federal University of Rio De Janeiro, Rio De Janeiro, Brazil, [2]Who Collaborating Centre, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [3]Lacen Rj, Noel Nutels Public Health Central Laboratory, Rio De Janeiro, Brazil

Dengue virus (DENV) is the most rapidly spreading mosquito-borne virus, and became the major public-health concern throughout tropical and sub-tropical regions of the world. There are 4 closely related and potentially co-circulating serotypes (DENV1, DENV2, DENV3 and DENV4). These serotypes are further divided into genotypes, which generally present characteristic geographical pattern that reflects the spatial dynamics of epidemics. Phylogenies of DENV4 delineate 4 major genotypes (Genotypes I; II; III and Sylvatic). Genotype II was the first detected in the Americas and responsible for all the epidemics reported until now. In Brazil, DENV 4, genotype II, was introduced in 1981 and remained the next 29 years without any occurrence, until 2010, when an epidemic was reported in the North region. After, the virus spread all over the country and reached Rio de Janeiro City (Southeast region) by the end of 2011. Nevertheless, evidences of introduction and circulation of genotype I, representing strains from Thailand, the Philippines, Sri Lanka, and Japan, has been reported in Brazil in 2008. The first detection of genotype I, occurred in Manaus city, (North region). Moreover, during our investigation of the DENV4 genotype II epidemic in Rio de Janeiro city, a DENV4 genotype I strain was also detected. The phylogenetic analysis of this strain, using full genome and partial glycoprotein E gene, demonstrates that it clusters with strains from Thailand, Salvador, and Cambodia, and not with the strains of Manaus, North region of Brazil. This suggests frequent introductions of DENV4 genotype I in Brazil, and also points Southeast region, as an important door for the introduction of DENV into South America. Thus, our study contributes to the knowledge of the phylogeographic dynamics of DENV in Brazil.
Financial support: BNI, FAPERJ, INBEB

Board No: 5 *Epidemiology, Modeling and Prediction*

BIOINFORMATICS STRUCTURE OF RECOMBINATION SITES AMONG STRAINS OF WESTERN SUBTYPE OF TICK-BORNE ENCEPHALITIS VIRUS

Y. Dzhioev[1,2], A. Paramonov[2], I. Kozlova[2,3], S. Tkachev[4], O. Suntsova[2], E. Doroschenko[2], O. Lisak[2], T. Demina[5], A. Borisenko[1,2], A. Lyapunov[2], I. Voitsekhovskaya[6], D. Růžek[7,8]

[1]Laboratory of Molecular Virology and Biotechnology, Institute of Biomedical Technology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [2]Laboratory of Molecular Epidemiology and Genetic Diagnostics, Fsbi "Scientific Center for Family Health and Human Reproduction Problems" Sb Rams, Irkutsk, Russian Federation, [3]Department of Mcrobiology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [4]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine Sb Ras, Irkutsk, Russian Federation, [5]Faculty of Veterinary Medicine and Biotechnology, Irkutsk State Academy of Agriculture, Irkutsk, Russian Federation, [6]Laboratory Adaptation Problems Biosystems, Institute of Biology of the Irkutsk State University, Irkutsk, Russian Federation, [7]Laboratory of Arbovirology, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, [8]Department of Virology, Veterinary Research Institute, Brno, Czech Republic

One of the leading factors of variation, evolution, adaptation and pathogenesis in viruses is recombination process. The existence of recombination in tick-borne encephalitis virus (TBEV) is still disputed. We present the results of bioinformatics analysis of recombination events in the genomes of 20 strains of the Western subtype TBEV. Programming techniques of recombination detection of software package RDP v4.14 (RDP, BootScan, Chimaera, Genecown, MaxChi, SiScan, 3Seq) were used. Only 6 programs (without 3Seq) significantly ($p < 0.5$) recorded recombination sites in three strains of TBE: Joutseno, AS33, 263. Five programs (without RDP) identified two recombination sites in strain Joutseno in the following positions of TBEV genome: BootScan: 943 -1702 and 7077-8554; Chimaera: 946-1702 and 7140-8558; Genecown: 888-1702 and 7137-8554; MaxChi: 946-1702 and 7137-8554; SiScan: 946-1702 and 7140-7983. RDP recorded one site in Joutseno: 6960-8554 and one site in strain AS33: 5522-8218, which other programs not confirmed. MaxChi recorded the large recombinant site in strain in position 2319-10023. Putative parental strains for recombinants are identified: for Joutseno in positions 943-1702; 7140-8558 and 7140-7983 – AS33 and Est3476.

In positions 7077-8554 of Joutseno strain BootScan identified strains 285 and Est3476, Genecown and MaxChi – CGI223 and Est3476, and RDP – strains KrM93 and Est3476 as parental. For recombinant strain 263 program MaxChi identified parent strains Salem and CGI223, and for strain AS33 program RDP identified parent strains Est3476 and KrM93.

The study was supported by the Russian Scientific Foundation (project № 14-15-00615).

Board No: 6 *Epidemiology, Modeling and Prediction*

MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPP. AMONG CHILDREN IN RURAL GHANA

D. Eibach[1], R. Krumkamp[1], H. Al-Emran[1], Y. Adu-Sarkodie[2], E. Tannich[3], J. May[1]

[1]Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Department of Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Molecular Parasitology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Introduction: The relevance of *Cryptosporidium* infections on the burden of childhood diarrhoea in endemic settings has been shown in recent years. This study describes *Cryptosporidium* subtypes among symptomatic and asymptomatic children in rural Ghana to analyse subtype-specific demographic, geographical, seasonal and clinical differences in order to implement appropriate control measure in this region.
Methods: Stool samples were collected from children below 14 years of age presenting with and without gastrointestinal symptoms at the Agogo Presbyterian Hospital in the rural Ashanti region of Ghana between May 2007 and September 2008. Samples were screened for *Cryptosporidium* spp. by PCR and isolates were classified into subtypes based on sequence differences in the gp60 gene. Subtype specific frequencies for age, sex, location, season and associations with disease symptoms have been calculated.
Results: *Cryptosporidium* infections were diagnosed in 65 symptomatic cases and 51 asymptomatic controls with males (mean age=0.85; sd 0.90) being significantly earlier infected than females (mean age=1.74; sd 2.36; p=0.006). Subtyping for 88 isolates revealed 51 *C. hominis* and 37 *C. parvum* strains. The three most frequently observed subtypes were IIcA5G3 (n=26, 29.6%), IbA13G3 (n= 17, 19.3%) and IaA21 (n=12, 13.6%), all known to be transmitted anthroponotically. Infections peak early during raining season with 67.9% and 50.0% of annual infections during the months May, April and June for 2007 and 2008 respectively, however no clusters of subtypes by time or place has been observed. The age-adjusted case-case comparison between *C. parvum* and *C. hominis* cases revealed no significant association for the symptom diarrhoea (OR=0.8; 95% CI: 0.3-2.6), yet *C. parvum* was more associated with vomiting (OR=2.5; 95% CI: 1.0-6.2)

Conclusions: Cryptosporidiosis in rural Ghana is characterized by seasonal anthroponotic transmission of strains typically found in Sub-Saharan Africa. The infection mainly affects young infants, with vomiting being one of the leading symptoms in *C. parvum* infection. Combining molecular typing and clinical data provides valuable information for physicians and public health authorities to apply preventive measures.

Board No: 7 *Epidemiology, Modeling and Prediction*

ENTEROHEMORRHAGIC *E. COLI* (EHEC) AND ATYPICAL ENTEROPATHOGENIC *E. COLI* (AEPEC) STRAINS OF SEQUENCE TYPE COMPLEX STC29 SHOW NO HOST SPECIFICITY IN IN SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ANALYSIS

I. Eichhorn[1], T. Semmler[1], A. Mellmann[2], D. Pickard[3], G. Dougan[3], L.H. Wieler[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany, [2]Institute for Hygiene, University Münster, Münster, Germany, [3]Wellcome Trust, Sanger Institute, Hinxton, Cambridge, United Kingdom

Multilocus sequence typing (MLST) of EHEC and aEPEC strains of the four most important non-O157 Serotypes (O26, O103, O111, O145) revealed that these strains represent a single sequence type complex (STC) – STC29. Within ST16, ST21, ST29 and ST113, the major sequence types of STC29, the mentioned serotypes cluster together. Furthermore both, aEPEC and EHEC, can be designated to the same STs. Hence STC29 displayes the close relationship between aEPEC and EHEC and their connected evolutionary background, even though they were characterized as different serotypes by now.

For this reason we have chosen 70 strains of STC29 for whole genome sequencing. The strains were obtained from different hosts, but mostly cattle (n=65), and belong to both pathotypes aEPCE and EHEC. The maximum common genome (MCG) of the 70 genomes was defined by identifying a set of conserved genes occurring in every of the considered genomes. The following single nucleotide polymorphisms (SNP) analysis of 1400 genes revealed 5107 SNPs. On the basis of these SNPs maximum spanning trees (MSTs) were generated to highlight the different characteristics of each strain (ST, pathotype and host). In addition the MSTs established from 5107 SNPs were compared to ones generated using the 48 SNPs that were published by Bletz et al. for STEC strains of serotype O26.

The MST that was generated using 5107 SNPs and representing the STs clarified that the MLST of seven housekeeping genes is a powerful method to display the relationship of *E. coli* strains. For the representation of the pathotype within this MST no tendency was recognizable, underlining the similarity between EHEC and aEPEC strains and their likely equal evolutionary background. The origin of the strains did not reveal any cluster formation, which rejects a host specificity of the strains and therefore supports the evidence for zoonotic transmission of EHEC and aEPEC strains.

Board No: 8 *Epidemiology, Modeling and Prediction*

SHARED POPULATIONS OF ESBL-PRODUCING BACTERIA IN HUMANS AND ANIMALS

J. Schmiedel[1,2], L. Falgenhauer[1,2], E. Domann[1,2], R. Bauerfeind[3], E. Prenger-Berninghoff[3], C. Imirzalioglu[1,2], T. Chakraborty[1,2]

[1]Institute of Medical Microbiology, Justus Liebig University Giessen, Gießen, Germany, [2]German Center for Infection Research, Partner Site Giessen Marburg Langen, Justus Liebig University Giessen, Gießen, Germany, [3]Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Gießen, Germany

Introduction and purpose: Multiresistant *Enterobacteriaceae* producing extended-spectrum ß-lactamases (ESBLs) are an emerging problem in human and veterinary medicine. The epidemiology of these bacteria is not completely understood. Zoonotic-like transmission from animals to human and vice versa has been proposed. In particular, transmission of strains or plasmids between humans and companion animals or horses is highly probable due to their close contact to each other. To specifically address this aspect, we initiated a comparative study of ß-lactamase- and ESBL-producing *Enterobacteriaceae* isolated from humans, companion animals and horses in central Hesse in Germany.

Methods: Clinical *Enterobacteriaceae* isolates (n = 361) from humans, companion animals and horses that were resistant against cefotaxime were selected for this study. Phenotypic detection of ESBL-producers was performed. Resistance genes *blaTEM*, *blaSHV* and *blaCTX-M* were initially characterized by PCR and sequencing. Plasmid-mediated quinolone resistance genes (PMQR), *blaOXA-1* and *blaOXA-48* were identified. *Escherichia coli* isolates were assigned to phylogenetic groups A, B1, B2 and D. Resistance gene combinations were analysed using Gene-E software.

Results: 316 (87.5 %) of the investigated isolates were confirmed to harbour an ESBL gene. Predominant ESBL subtypes in human and animal isolates were CTX-M-15 (49.3 %) and CTX-M-1 (25.8 %). Resistance gene combinations common to human and animal isolates were found. These included CTX-M-1/TEM-1 or CTX-M-15/OXA-1/aac(6`)Ib-cr in *Escherichia coli*. Resistance genes present only in animals were blaCTX-M-2, (found almost exclusively in equines), whereas the carbapenemase OXA-48 was detected in companion animal isolates, only.

The most frequent ESBL-producing species was *Escherichia coli*, followed by *Klebsiella pneumoniae* and *Enterobacter cloacae*. *Klebsiella pneumoniae* was more frequently detected in dog isolates. Investigation of *Escherichia coli* phylogenetic groups revealed underrepresentation of group B2 within the animal isolates.

Conclusion: Isolates from human, companion animals and horses shared several characteristics regarding presence of ESBL, PMQR and combination of different resistance genes. These results suggest the idea of active transmission and dissemination of multiresistant *Enterobacteriaceae* or their respective resistance conferring genes between human and animal populations. The study strongly supports the One Health concept which expands interdisciplinary collaborations and communications in all aspects pertaining to the health care for humans and animals.

Board No: 9 *Epidemiology, Modeling and Prediction*

COMPLETE SEQUENCE OF A PLASMID FROM A BOVINE MRSA HARBOURING ANTIMICROBIAL AND HEAVY METAL RESISTANCE GENES IN ADDITION TO PUTATIVE VIRULENCE GENES

A.T. Feßler[1], Q. Zhao[2], K. Kadlec[1], Y. Wang[2], W. Ziebuhr[3], J. Shen[2],S. Schwarz[1]

[1]Institute of Farm Animal Genetics, Friedrich Loeffler-Institut (-), Neustadt Mariensee, Germany, [2]College of Veterinary Medicine, China Agricultural University, Bejing, China, [3]Institut Für Molekulare Infektionsbiologie, Universität Würzburg, Würzburg, Germany

During previous studies, MRSA isolates with elevated apramycin minimum inhibitory concentrations of ≥32 mg/L have been detected, all of which harbored the apramycin resistance gene *apmA*. This gene was mainly located on multiresistance plasmids conferring resistance to seven classes of antimicrobial agents. The aim of this study was to sequence one of these plasmids completely and analyse it for its structure and organisation.
Plasmid pAFS11, from which an 11-kb fragment had been sequenced previously, was chosen for sequence analysis. Sequencing was performed using the Illumina Hiseq 2500 (Berry Genomics Company, Beijing, China). Gap closure between the different contigs was done by PCR and sequencing of the amplicons.
The 49,192-bp plasmid pAFS11 harboured the apramycin resistance gene *apmA*, two copies of the macrolide/lincosamide/streptogramin B resistance gene *erm*(B), the kanamycin/neomycin resistance gene *aadD*, the tetracycline resistance gene *tet*(L) and the trimethoprim resistance gene *dfrK*. The *apmA* gene was located upstream of one of the *erm*(B) copies. The two *erm*(B) genes were located in the same orientation, but 5,790 bp apart from each other. The remaining three resistance genes, *aadD*, *tet*(L) and *dfrK*, were located on a 6,388-bp segment which was bracketed by two copies of IS*431* located in the same orientation. This segment was inserted into a *repU* plasmid replication gene. Upstream of the right-hand IS*431* copy, another truncated *rep* gene, the cadmium resistance operon *cadDX* and an IS*257R1*-like transposase were found. Downstream of the left-hand IS*431*, the copper resistance genes *copA* and *mco* as well as a complete *ica*-like gene cluster, were detected. This *ica* gene cluster was composed of four genes which showed only limited homology to other staphylococcal *ica* genes. The area upstream of the *ica*-like cluster contained an IS*257* element, a *parA* gene and another *rep* gene.

The finding of five different antibiotic resistance genes co-located on the same plasmid together with heavy metal resistance genes and an *ica*-like gene cluster, whose role in biofilm formation is currently under investigation, is alarming. With the acquisition of this plasmid, antimicrobial multi-resistance and potential virulence properties may be co-selected and acquired via a single horizontal gene transfer event.

Board No: 10 *Epidemiology, Modeling and Prediction*

VIRULENCE CHARACTERISTICS OF FOOD AND HUMAN STRAINS OF *LISTERIA MONOCYTOGENES* SEROTYPE 1/2C

R. Karpišková[1], T. Gelbicova[2], I. Kolačková[1]

[1]Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic,
[2]Department of Experimental Biology, Masaryk University, Brno, Czech Republic

Currently, internalin A has been considered as a molecular marker for evaluating of a potentially attenuation virulence of *L. monocytogenes*. Particularly strains of serotype 1/2c, which rarely cause listeriosis in humans, have been associated with a frequent occurrence of a truncated internalin A. In the Czech Republic, only one case of human listeriosis was noted to be caused by serotype 1/2c. The study objective was to compare virulence characteristics of strains of *L. monocytogenes* serotype 1/2c isolated from neonatal listeriosis and food strains of the same serotype and pulsotype. The strains were characterized by serotyping, macrorestriction analysis after digestion with the restriction enzyme *AscI*, PCR detection of virulence genes, RFLP analysis of *inlA*, and sequencing of the *inlA* gene. All genes playing a significant role in pathogenesis of *L. monocytogenes* (*prfA*, *hlyA*, *plcA*, *plcB*, *actA*, *inlA*, *inlB*) together with *inlC* and *inlJ* were detected in our tested strains. None strains possessed *llsX* gene. The strains of serotype 1/2c, pulsotype 1 were identified to be of RFLP profile 4 which may be associated with the production of the truncated internalin A. However, we detected quite new point mutations of *inlA* leading to a premature stop codon (PMSC) only in strain isolated from the neonatal case of listeriosis. The strains of serotype 1/2c (pulsotype 1) isolated from foods contained no mutations in the sequenced region of the *inlA* gene leading to a PMSC. The results show that detection of PMSCs in *inlA* may not mean attenuated virulence of *L. monocytogenes*, but may reveal additional differences between tested strains.

This study was carried with financial support of NAZV KUS QJ1210300, CEB project CZ.1.07/2.3.00/20.0183 and and of the MEYS of the CR under the NPU I program.

Board No: 11 *Epidemiology, Modeling and Prediction*

OCCURRENCE AND DYNAMICS OF SHIGATOXIN-PRODUCING *ESCHERICHIA COLI* IN GERMAN PETTING ZOOS

P. Grüning[1], A. Drees[1], T. Bauer[1], A. Campe[2], J. Verspohl[1], A. Mellmann[3], A. Fruth[4], G. Gerlach[1], P. Valentin-Weigand[1]

[1]Institute for Microbiology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [2]Institute for Biometry, Epidemiology and Information Processing, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [3]Institute of Hygiene, University Hospital Muenster, Muenster, Germany, [4]National Reference Centre for Salmonella and Other Bacterial Enterics, Robert Koch Institute, Wernigerode, Germany,

Shigatoxin-producing *Escherichia coli* (STEC) can cause bloody and non-bloody diarrhea as well as hemolytic uremic syndrome (HUS), especially in children. Most STEC infections in humans are associated with consumption of raw milk or raw meat. Another source of infection is the close contact to ruminants. Therefore, we performed two studies in 2009 and 2011/2012 to evaluate the occurrence of STEC in German petting zoos. In the first study 32 petting zoos were examined. In 27 of the tested zoos STEC could be isolated, mainly from goats and sheep. More than 30 different serotypes were detected, 11 of these were identical to those listed in the HUSEC-strain collection (Mellmann et al. 2008). Nine of the previously positive zoos also participated in the second study 2011/2012. The petting zoos were sampled quarterly over a period of one year. All tested zoos remained STEC positive, and 26 different serotypes were isolated. In some of the zoos, several serotypes were detected during the whole time period. Summarizing, STEC occurs in German petting zoos with a high diversity of serotypes, also including serotypes that are identical to those of the HUSEC-strain collection. Repeated isolation of some serotypes over the whole time period suggests that STEC is endemic in petting zoos.

Board No: 12 *Epidemiology, Modeling and Prediction*

ECOLOGICAL NICHE MODELING OF GEOGRAPHIC DISTRIBUTION OF *BACILLUS ANTHRACIS* AND RISK OF ANTHRAX DISEASE IN AFRICA

B.E. Isabirye[1], A. Mayamba[2], E. Zziwa[1], C. Nakato[2], I. Rwomushana[1]

[1]Sustainable Agriculture, Food and Nutrition (SAFN), Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), Kampala Uganda, Uganda, [2]National Agricultural Research Laboratories, Naro Uganda, Kampala, Uganda

Keywords: Africa, Anthrax, Ecological Niche, potential distribution, Zoonosis

Background: Anthrax is one of the seven major neglected zoonotic diseases in Africa. The disease is responsible for enormous economic losses as well as considerable human morbidity, especially in endemic areas. Despite this, the ecology and distribution of the causative agent, *Bacillus anthracis*, is poorly understood. At the continent level, there is glaring paucity of knowledge on the potential environments conducive for spore survival and subsequent disease outbreaks.

Methods: We modeled the ecological niche of B. anthracis and risk of Anthrax using Maximum Entropy (MaxEnt), Bioclimatic analysis and prediction system (BIOCLIM) and a genetic algorithm (GARP). Model inputs included climatic (temperature and rainfall), topographic (slope and aspect) and records of Anthrax outbreaks in Africa.

Results: The three model performances were better than random expectations, statistically significant ($p < 0.05$), and with ROC/ AUC scores ranging from 71.8% (BIOCLIM) to 88.1% (GARP). The predicted total land area of highly suitable environments ranged from 5.713 million Km2 (18.9%) to 20.044 million Km2 (66.3%). Qualitative assessments of historical records of Anthrax outbreaks across Africa confirm model predictions. Conducive environments for the sporulation and viability of B. anthracis corresponded to a range of temperature, precipitation and topographic regimes in the semi-arid, sub-humid and dry to moist subtropical mid-latitude conditions in eastern and southern Africa. West and Central Africa, and the extreme North Africa presented marginal, while the much of the Sahel and Congo basin were predicted to be of low suitability.

Conclusions: Results of the three models indicated extensive spatial suitability for *B. anthracis* and high risk of Anthrax in Africa. Given the current status of Anthrax in Africa (neglected zoonosis), our results should be important in efforts geared at prevention or eradication of the disease. Our findings will also aid in anticipating outbreaks and differentiating endemic/natural outbreaks from other forms outbreaks.

Board No: 13 *Epidemiology, Modeling and Prediction*

AREAS AT RISK OF ZOONOSES – CONSIDERING ECOLOGICAL KNOWLEDGE OF BOTH PATHOGEN AND VECTOR IN MODELLING VECTOR-BORNE DISEASES

A. Jaeschke[1,2], S.M. Thomas[1], N.B. Tjaden[1], C. Beierkuhnlein[1]

[1]Biogeography, University of Bayreuth, Bayreuth, Germany, [2]Biogeographical Modelling, University of Bayreuth, Bayreuth, Germany

Current developments in the spread of arthropods as well as pathogens in Europe emphasize the importance and urgency of well-informed projections concerning the expectable future development. The basic idea behind the identification of current and future areas at risk of zoonoses is the combination of ecological knowledge covering the pathogen and the vector; and where possible the host.
Within a pilot project of the German Research Platform for Zoonoses ('Zoonosis RISKTOOL') we aim to develop current and future risk maps for Dengue and West Nile with special focus on the cold tolerance of the vector and the extrinsic incubation period (EIP) of the pathogen. Based on these examples we further develop a Zoonosis RISKTOOL allowing a general application of the approach for other zoonoses.
Modelling is performed with the open-source statistic software R and the implemented package biomod2. Biomod2 allows the use of an ensemble of different modelling algorithms and hence a reduction of the uncertainty by using only one algorithm. The RISKTOOL provides all necessary steps: variable selection, model calibration and validation, implementation of ecological knowledge as well as current and future projections of the distribution of zoonoses.
As study vectors we used Aedes albopictus and Aedes japonicus. The cold tolerance of both vectors was tested experimentally. For Dengue we further estimated the EIP for different temperature values from the literature. Both ecological aspects act as a filter for the distribution models, i.e. we acknowledge ecological constraints.
The EIP is displayed as a temperature-duration relationship, which means that with an increase in temperature the duration of the EIP becomes shorter and hence the risk for a pathogen transmission increases. The experiment on the cold tolerance revealed that the combination of the minimum temperature and the duration of the exposure is the most important variable constraining the reproduction and hence the long-term establishment of the vector.

Assessing the risk of a spread of zoonoses requires the combination of projections for vector and pathogen as well as the inclusion of ecological constraints to yield a more realistic estimation. Prospectively, the integration of the host, especially for further zoonoses, will be necessary.

Board No: 14 *Epidemiology, Modeling and Prediction*

SEROPREVALENCE OF *COXIELLA BURNETII* AMONG FOREST WORKERS

S.F. Fischer, D. Kömpf

Landesgesundheitsamt Baden Württemberg, Konsiliarlabor Für Q Fieber, Stuttgart, Germany

Introduction: Q fever is a widespread zoonosis caused by the obligate intracellular and highly pathogenic bacterium *Coxiella burnetii* (*C. burnetii*). Up to now little is known about the prevalence of this infectious disease. Therefore a study was performed in a population potentially being at high risk for getting Q fever: a group of forest workers as well as a control group was analysed for antibodies against*C. burnetii*. Furthermore possible risk factors were determined.
Methods: A cross-sectional study with a total of 605 participants (forest workers and control group) of the North Rhine-Westphalian "Landesbetrieb Wald und Holz" was conducted. Risk factors were ascertained by a specific questionnaire. *C. burnetii* specific tests were done: first we screened the sera for IgM and IgG antibodies against phase II antigen using an Enzyme Linked Immunosorbent Assay (ELISA). Positive samples were confirmed by indirect immunofluorescence test (IFT) to determine exact antibody titers.
Results: Altogether 605 sera were tested whereof 33 showed specific antibodies against *C. burnetii* (5,5%). Looking at the group of forest workers we could show that 6,0% showed positive serology. In comparison only 4,2% in the control group (no forest workers) showed positivity for antibodies against *C. burnetii*. Specific risk factors for Q fever were identified by a questionnaire.
Discussion and conclusion: To conclude: in this study we could show that the prevalence for *C. burnetii* in forest workers was higher than in the control group. For Q fever the results for the persons at risk (forest workers) confirmed the expectations while it was surprising that even the control group presents a relative high prevalence. In the future the risk factors have to be analysed in more detail.
So far we have to point out that knowledge on the distribution of Q fever in Germany is still poor. Further investigations are necessary to get a more detailed insight into this infectious disease. Strategies and methods have to be improved to support the public health system to detect possible outbreaks and to protect the population from this illness.

Board No: 15 *Epidemiology, Modeling and Prediction*

RICKETTSIA SP. ON THE CANARY ISLANDS, SPAIN

L. Kurzrock[1], R. Oehme[2], P. Sebastian[2], S. Lorentz[3], T. Naucke[3,4], U. Mackenstedt[1]

[1]Department of Parasitology, University of Hohenheim, Stuttgart, Germany, [2]State Health Office, Baden Wuerttemberg, Stuttgart, Germany, [3]Parasitus Ex e.v., Parasitus Ex e.v., Niederkassel, Germany, [4]Laboklin, Laboklin, Bad Kissingen, Germany

Rickettsiosis is still an emerging zoonotic disease challenging scientists around the world. Little is known about the parameter that influences the distribution of *Rickettsia*. Ecological factors as temperature, precipitation and host and vector density were taken under consideration of having an impact on rickettsial prevalence. To contribute to the knowledge about the distribution, this study aimed to investigate rickettsial bacteria prevalence on the Canary Islands, Spain. In 2010 and 2012 1.490 ticks of the species *Rhipicephalus sanguineus* and *Rh. turanicus* as well as 291 fleas of the species *Ctenocephalides felis* and *Echidnophaga gallinacea* were collected from dogs on five islands (Lanzarote, Fuerteventura, Gran Canaria, Tenerife and La Palma). Rickettsial DNA was identified by RealTime-PCR targeting the citrate synthase-encoding gene (gltA). Prevalences in ticks ranged from 14 % to 56 % depending on year, island and isolation spot with a mean value of 20.3 % in 2010 and 32.8 % in 2012. In investigated Ct. felis prevalences ranged from 25 % to 38 % (mean value 30 %). All tested *Ec. gallinacea* were negative on rickettsial DNA. *Rickettsia*isolated from ticks were so far identified as *R. massiliae* in 93 %. In fleas we detected *R. felis* up to date in 81 %. Both species are known to be pathogen for humans.
As we could show, *Rickettsia*is highly present in Canarian ticks and fleas and therefore a potential risk for human health.

Board No: 16 *Epidemiology, Modeling and Prediction*

THE ZOONOSIS RISKTOOL: MODELLING FRAMEWORK

N.B. Tjaden[1], A. Jaeschke[1,2], S. Thomas[1], C. Beierkuhnlein[1]

[1]Department of Biogeography, University of Bayreuth, Bayreuth, Germany,
[2]Department of Biogeographical Modelling, University of Bayreuth, Bayreuth, Germany

The Zoonosis RISKTOOL is currently being developed in a Pilot Project under the umbrella of the German Research Platform for Zoonoses. We aim to create a modeling framework for the continental to regional scale risk assessment of vector-borne diseases based on environmental and ecological drivers. We combinine classic correlative species distribution models for vector species with process-based modules, incorporating additional factors such as the temperature-dependence of the Extrinsic Incubation Period of the pathogen or winter survival of the arthropod vectors. Written in the well-established open-source statistical scripting language R, the RISKTOOL will be free to anyone to use, share, and modify according to their individual research requirements. Here we present in detail the full structure of our modelling framework.

Board No: 17 *Epidemiology, Modeling and Prediction*

TICK-BORNE ZOONOTIC BACTERIAL DISEASES IN KAZAKHSTAN

N. Turebekov[1], A. Dmitrovsky[1], R. Yegemberdieva[1], Z. Shapieva[2], L. Yeraliyeva[1], S. Frey[3], J. Zinner[3], S. Essbauer[3]

[1]Department for Infectious Diseases, Kazakh National Medical University, Almaty, Kazakhstan, [2]Department of Parasitology, Scientific and Practical Center of Sanitary and Epidemiological Expertise and Monitoring, Almaty, Kazakhstan, [3]Department of Virology and Rickettsiology, Bundeswehr Institute of Microbiology, Munich, Germany

In the Republic of Kazakhstan there are at least 47 species of ticks described belonging to e.g. Ixodes, Dermacentor, Hyalomma and Rhipicephalus species. Ticks carry a variety of bacterial zoonotic pathogens such as Rickettsia sp., Borrelia sp., Fransicella sp. and Coxiella sp.. So far in Kazakhstan for these four groups of bacteria there is only limited knowledge on distribution and the impact on disease in humans. There exist some data on endemic areas (East (Oskemen), North (Petropavlovsk) and West (Oral) Kazakhstan, Kyzylorda and Almaty regions) for some of these tick-borne bacterial, zoonotic pathogens but exact annual human cases the circulating range of species and genotypes of e.g. Borrelia, Rickettsia, Francisella and Coxiella are unknown. For example for tularemia, unfortunately, despite the relatively good knowledge on natural foci, modern laboratory diagnosis of this infection in humans is lacking. Comprehensive studies on the current status of tularemia and Q fever in Kazakhstan, with special focus on pathogen reservoirs and vectors, the sources of infection are missing. Further for borreliosis and rickettsiosis, there was no extensive research on the prevalence in the Republic of Kazakhstan performed. Clinical-epidemic features of tick-borne borreliosis and North-Asian tick-borne rickettsiosis have to be studied in more detail in Kazakhstan. In summary, further data and modern laboratory technique are needed to improve the knowledge and take countermeasures against the diseases caused by these four bacterial agents Therefore we initiated a project to perform serological studies in patients with fever of unknown etiology and a surveillance of these tick-transmitted bacterial pathogens in ticks. Further we aim to implement Standard Operating Procedures for the laboratory diagnosis of the mentioned tick-borne bacterial diseases in Kazakhstan.

This project is funded by the German Federal Foreign Office in the framework of the *German Partnership Program for Excellence in Biological and Health Security*.

Board No: 18 *Epidemiology, Modeling and Prediction*

SOURCE ATTRIBUTION OF FOODBORNE ESBL- *E. COLI* USING DATA FROM THE RESET CONSORTIUM

L. Valentin, H. Sharp, B. Appel, A. Käsbohrer

Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

Extended-spectrum beta-lactamases (ESBL) producing *E. coli* are resistant against a wide spectrum of ß-lactam antibiotics, including 3rd- and 4th-generation cephalosporins. In addition, such bacteria are often resistant to other antimicrobial classes. Furthermore, the underlying genetic information can be potentially transferred inter- and intra-species which may contribute to the public health risk.

There is an ongoing discussion about the origin of ESBL-producing *E. coli* in humans and the role of animal food sources. Within the German RESET project (www.reset-verbund.de) several studies investigated the prevalence of ESBL-producing *E. coli* in animal and human populations and characterized the isolates using phenotypic and genotypic techniques. Based on these study results, we estimated the possible contribution of different animal sources (broiler, fattening pigs, and cattle) to the colonization of the general population with ESBL-producing *E. coli*. In addition, we considered in one approach hospital acquired cases as 'source' to investigate the correspondence with ESBL–subtypes found in the community. Our model approach is based on Tine Hald's Salmonella source attribution model using microbial subtyping data. Information on ESBL-genes, phylogenetic groups and the antibiotic resistance pattern were incorporated to define the subtypes considered in the model.

Our results show that – on the basis of ESBL-genes and phylogenetic groups - several subtypes found in human cases colonized with ESBL-producing *E. coli* can be explained by animal food sources. If only cattle, pigs and broilers are considered as sources, around half of the human findings might be explained by these sources. If information on antimicrobial resistance pattern is included for subtype definition, the number of findings in the human community, which can be explained by any of the investigated animal sources decreased considerably. This is due to the huge variation of subtypes found in each of the populations. As expected, quite a proportion of all subtypes found in the community match with those found in hospital acquired cases, if included as 'source' to the model. This indicates that these human populations share a considerable number of subtypes and that these animal sources may explain only a part of the findings of ESBL-genes in humans.

This approach can be further developed to assess the role of the different reservoirs for exposure of humans with ESBL-producing *E. coli* or ESBL-genes.

Board No: 19 *Epidemiology, Modeling and Prediction*

THE GENETIC STRUCTURE OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* STRAINS IN GERMANY

B. Walther[1], A. Lübke-Becker[1], I. Stamm[2], S. Vincze[1], A.G. Brandenburg[2], L.H. Wieler[1], T. Semmler[1]

[1]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [2]Vet Med Labor GmbH, Division of Idexx Laboratories, Ludwigsburg, Germany

In dogs and cats, infectious diseases due to multidrug-resistant methicillin resistant *Staphylococcus pseudintermedius* (MRSP) involve mostly wound, skin or ear infections. Nosocomial transmission in veterinary clinics is reported as well as severe cases of disease in human patients. Since 2005, a tremendous epidemic spread of MRSP belonging to sequence type (ST)71 appeared first in Europe and later in other parts of the world, especially Asia.

To get a deeper insight into the population structure of MRSP-ST71, we comparatively analyzed a set of 100 MRSP isolates by pulsed-field gel electrophoresis (PFGE) representing various geographic origins in Germany. In contrast to the common homologies of *Staphylococcus aureus* isolates sharing the same ST, PFGE-analysis using Bionumerics® (Applied Maths, Belgium) revealed a remarkable range of different pattern associated with MRSP-ST71, an observation which was reported for Asian ST-71 isolates as well. Whole genome sequencing (Illumina MiSeq) and comparative genomics were performed on 11 MRSP-ST71 isolates representing diverse origins. A global alignment of whole genome sequences (WGS) together with data of a recently published reference genome (MRSP-ST71 E140) using progressive MAUVE revealed sequence blocks which were present in all strains, but also regions which harbor more variable gene content.

A maximum likelihood tree (RAxML) based on the maximum common genome (MCG) of 12 MRSP-ST71 (11 isolates and E140) together with the WGS data obtained for three susceptible *S. pseudintermedius* (MSSP) belonging to three distinct ST's representing out-groups was calculated. As a result, the novel multi-locus sequence typing (MLST)-scheme published for *S. pseudintermedius* is supported by WGS.

Interestingly, the staphylococcal cassette chromosome *mec* (SCC*mec*)-element conferring methicillin resistance belongs to type II-III in 10 of the 11 genomes, but a singel isolate harbored a putative novel variant.

Board No: 20 *Epidemiology, Modeling and Prediction*

CHALLENGES OF A MULTI-SECTORAL INFORMATION INTEGRATION FOR ZOONOTIC DISEASE SURVEILLANCE

A. Wendt, L. Kreienbrock, A. Campe

Department of Biometry, Epidemiology and Information Processing, Who Centre for Research and Training in Veterinary Public Health, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Against the background of One Health there are growing demands to improve the surveillance of zoonotic diseases by integrating information from humans and animals. Regarding this interdisciplinary approach and the high costs of collecting new data, a feasibility study was set up to evaluate, if existing data on zoonoses from different sources in Germany could be used in collaboration and whether this would improve the prevention and control of zoonotic diseases.
Therefore, an inventory of 17 databases, holding routinely collected zoonotic disease information on national level, was taken. For this purpose a questionnaire was developed to enable a systematic description of the data sources in general, their content, format, transparency for secondary use and some quality indicators. The suitability of the existing data was evaluated for a number of selected surveillance purposes, most of them regarding early warning and prompt detection.
Given the current state of data situation and considering different surveillance purposes it became apparent that data integration is hardly possible to realize. Reasons for this are different data collection methods, lacking data depth or lacking timeliness. Additionally, due to the federal structure of Germany, even data that are detailed and timely such as laboratory diagnoses are not available for a joint use. These laboratory data are scattered through a wide variety of different databases from different institutions. Hence, they are neither consistent, nor is there a one-stop shop for access on a nationwide level, for the time being.
Despite of these challenges hindering a joint analysis, we also identified needs of different stakeholders to share information rather than data on zoonotic disease events. Therefore, interdisciplinary structures for a reliable and binding communication have to be established over time in order to enable the timely sharing of information on zoonotic diseases from a local to national level between the partners from human and animal health as well as the food safety sector.

This presentation will give insight into our experiences, regarding challenges and needs of a multi-sectoral information integration and present a tool for a systematic description of already existing data sources, in order to enable a future secondary use of the data within these sources.

Board No: 21 *Epidemiology, Modeling and Prediction*

SPREAD HEMORRHAGIC FEVERS AND SURVEILLANCE PROBLEMS FOR THEM IN THE REPUBLIC OF KAZAKHSTAN

L. Yeraliyeva[1], R. Yegemberdieva[2], A. Dmitrovsky[2], Z. Shapieva[3]

[1]Department of Children Infectious Diseases, Kazakh National Medical University, Almaty, Kazakhstan, [2]Department of Infectious Diseases, Kazakh National Medical University, Almaty, Kazakhstan, [3]Department of Parasitology, Scientific and Practical Center of Sanitary and Epidemiological Expertise and Monitoring, Almaty, Kazakhstan

In the Republic of Kazakhstan (RK), currently, two haemorrhagic fever (HF) are officially registered. Those are Crimean-Congo HF (CCHF) and HF with renal syndrome (HFRS). Studies of arbovirus infections, conducted by the research team lead by Karimov throughout 60-80s, demonstrated the presence of circulation of other arboviruses as potential etiologic agents of hemorrhagic fevers. The aim of the present study is to analyse the incidence of HF in the RK, as well as the dynamics of change of endemic areas, the existing problems of public health surveillance and prospects of their research.

Using, analysis methods of epidemiology and laboratory diagnostics (ELISA, PCR, IFT), we analysed official data reports.

Studies of tick population on the matter of CCHF virus infestation showed an expansion of endemic areas. Over the past 25 years, 519 cases of CCHF were registered. The conducted serological inspection of 265 individuals exposed to tick-bites in the CCHF endemic areas, suggested the presence of antibodies to the virus in 12.7 % cases. The examination of 130 patients with obscure fever also showed the presence of antibodies to CCHF in 30.8 % of cases.

Registered HFRS appeared since 2000 in West Kazakhstan region where the natural focus of HFRS was formed, actively expanding to the south. In the period from 2000 to 2005, the number of cases increased from 3 to 85 (morbidity rates of 0.49 - 14.52, respectively).The examination of 148 people living in HFRS endemic areas identified high prevalence rate (28.4%) of hantavirus antibodies presence.

Thus, currently there are two types of HF formally recorded in Kazakhstan-CCHF (in three areas: Kyzylorda, South Kazakhstan and Zhambyl) and HFRS (in one area: West Kazakhstan).

At the same time, the inspection of people with tick bites, patients with fevers of unknown aetiology and of the population in an endemic area, indicates wider dissemination of the infection, as well as absence of registered cases of other types of HF, despite the circulation of viruses. This points out the necessity to improve the existing surveillance system, to expand the range of diagnosis and research of viruses' genotypes.

Board No: 22 *Pathogen-Cell Interaction and Immunity*

EXPERIMENTAL TRIAL WITH HEAT SHOCKED PROTOSCOLECES EXTRACT AS VACCINE CANDIDATE FOR PROTECTION AGAINST HYDATID DISEASE

H.M. Alsakee[1], H. Hassan[2], T. AL-Hadithi[3]

[1]Microbiology, Hawler Medical University, Erbil, Iraq, [2]Department of Biology, University of Kirkuk, Kirkuk, Iraq, [3]Department of Community Medicine, Hawler Medical University, Erbil, Iraq

Keywords: Echinococcus granulosus, vaccination, heat shock protein 70

Background and objectives: Cystic echinococcosis is widely distributed throughout the world and is still an important public health challenge in many countries of the world. The present study is an experimental trial to use hydatid antigens derived from viable protoscoleces cultivated at 37°C and 45°C for four hours, as vaccine candidate for protection against hydatid infection.

Materials and methods: Three groups (12 each) of balb/c mice aging 6-8 weeks old were immunized with hydatid antigens exctrated from *Echinococcus granulosus* protoscoleces exposed to 37°C, 45°C as well as partially purified hydatid antigens containing heat shock protein 70 of 30 µg, 60 µg and 90 µg , administered with adjuvant and without adjuvant.Those mice were then experimentally infected with 2000 viable protoscoleces and killed three months later. Three further groups (12 each) of mice were used as positive, negative and adjuvant controls.

Results: The results showed that crude antigens from protoscoleces exposed to 37°C conferred non significant immunity with protection and reduction rates ranged from 0% - 25% and 77.69% - 98.38% respectively.In mice receiving crude antigens from protoscolecec exposed to 45 °C, the protection and reduction rates ranged from 0% - 66.66% and 94.62% - 98.92% respectively. Purified antigen from 45°C exposed protoscoleces conferred significant immunity with absolute protection observed in mice immunized with 60 µg and 90 µg of antigen combined with adjuvant. Immunological parameters (anti-hydatid antibody titer and lymphocyte transformation %) showed negative correlation with the number of cysts. Assessment of renal and liver functions showed non significant differences ($p > 0.05$) in comparison with non-immunized mice of negative control group.

Conclusion: This study has suggested that purified hydatid antigen containing heat shock protein 70 confer high level of protection against hydatid infection in mice.

Board No: 23 *Pathogen-Cell Interaction and Immunity*

PERMISSIVENESS OF BOVINE EPITHELIAL CELLS FROM LUNG, INTESTINE, PLACENTA AND UDDER FOR INFECTION WITH *COXIELLA BURNETII*

K. Bonkowski[1], K. Sobotta[1], I.D. Jacobsen[2], C. Menge[1]

[1]Institute of Molecular Pathogenesis, Friedrich Loeffler Institut, Jena, Germany,
[2]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology / Hans Knoell Institute, Jena, Germany

Ruminants are the main source of human infections with *Coxiella burnetii* (*C. b.*), the causative agent of Q fever. Infected animals shed high numbers of *C. b.* by milk, feces, and birth products. Aims of the study are to analyze the interactions of *C. b.* with the bovine host (1) at the entry site (lung epithelium) which imprints on the initiated host immune response and (2) in epithelial cells of gut, udder and placenta that determine the quantity of pathogen excretion. The immune response of the host cells and its modulation by the bacteria are investigated at the transcriptome and proteome level. Furthermore, peculiarities in the various host cells' metabolisms will be identified as to their role in the support of bacterial replication.

Epithelial origin of several bovine cell lines (PS [udder], FKD-R 971 [small intestine], BCEC12/T2 [maternal placenta], F3 [fetal placenta], BEL-26 [lung]) was confirmed by immunofluorescence studies and western blot analyses. Epithelial cell lines responded to lipopolysaccharide (LPS) stimulation by differential expression of cytokines and chemokines. In general terms, Th1-related cytokines (IL1-β, IL-8 and TNF-α) were highly expressed accompanied by a comparably weak Th2 response (MCP1, IL-6). To study permissiveness for bacterial invasion and replication, cell lines were inoculated with C. b. strain "Nine Mile phase II clone 4" and "Nine Mile phase I RSA 493" at different cultivation conditions. Cell viability was evaluated by LDH (lactate dehydrogenase) and MTT (methyl-thiazolyl tetrazolium) assays. Invasion and replication of *C. b.* were quantified by real-time PCR and microscopic studies. Bovine epithelial cell lines exhibited different permissiveness to *C. b.*while maintaining cell viability with udder cells allowing for the highest invasion and replication rates.

Subsequent host cell proteome analyses will aim at identifying molecules and metabolic pathways of host cells determining *C. b.* replication at the cellular and duration of infection at the animal level to unveil the immunobiology of this zoonotic pathogen in its host.

Board No: 24 *Pathogen-Cell Interaction and Immunity*

CHARACTERIZATION OF COWPOX VIRUS HOST RANGE FACTOR P28 IN MACROPHAGES

D. Bourquain, E. Richter, A. Nitsche

Centre for Biological Threats and Special Pathogens 1, Robert Koch Institute, Berlin, Germany

The increasing number of cowpox virus (CPXV) infections in Europe and reports on emerging human monkeypox outbreaks in Africa and North America remind us that – more than 30 years after the eradication of smallpox virus – zoonotic poxviruses may pose an emerging health threat to humans. The unique properties of host range, pathogenesis and the potential for host-to-host spread of each poxvirus are influenced by its individual portfolio of so-called host range genes. However, only a handful of these host range genes have been functionally characterized. In this study, we aimed at characterising the functions of the poxviral host range gene p28.

The p28/N1R RING zinc-finger protein is a 28 kDa protein which is highly conserved among orthopoxviruses like variola virus and CPXV. P28 is expressed early during infection and localizes to cytoplasmic virus factories. Functionally, p28 was described first as a virulence factor essential for ectromelia virus (ECTV) infection of mice. Furthermore, it acts as a host range factor and extends the *in vitro* host range of ECTV to macrophages. The significance of p28 as a host range factor in other poxviruses is largely unknown, although in a previous study we could show that p28 extends the host range of CPXV *in vitro*. A p28 knockout mutant of CPXV exhibited a reduced replication efficiency on macrophage cell lines, rat peritoneal macrophages and human PBMC-derived macrophages. Similar to ECTV, the attenuation was specific for cells of the monocyte/macrophage lineage.

The mechanism by which p28 extends the host range of ECTV and CPXV is still unknown. In this study, we established heterologous expression of the CPXV p28 protein in macrophages via transient transfection of synthetic p28-mRNA and analysed its capability to overcome the replication deficiency of vaccinia virus or recombinant CPXV lacking p28 in these cells. To further elucidate the mechanism of host range extension by p28, we compared the effects of different truncated p28 variants expressed heterologously which lacked different functional domains.

Board No: 25 *Pathogen-Cell Interaction and Immunity*

ESTABLISHMENT OF A NEW *IN VITRO* INFECTION MODEL FOR CAMPYLOBACTER JEJUNI INFECTIONS IN CHICKEN

A. Bruchmann, D. Schulte, A. Dreusch, W. Rudy

Research and Development, Micromol GmbH, Karlsruhe, Germany

Campylobacter represents the most commonly reported zoonotic pathogen leading to gastrointestinal disease in Europe [1]. Hereby the main infection source for human is undercooked poultry meat [2]. While chicken are asymptomatic carriers of the pathogen, in human infections can lead to bloody diarrhea, nausea, abdominal pain and fever and sometimes severe neurological diseases.
To reduce human Campylobacter infections, the incidence in chicken must be reduced. Therefore the need of preventing agents other than antibiotics is obvious. Until now, no effective intervention exists that reduces Campylobacter load in poultry [3].
In this regard, we developed an *in vitro* assay based on a newly developed chicken enterocyte cell line having the potential to investigate the ability of different compounds to reduce the infection in chicken. In this context the inhibitory function of a series of compounds has already been successfully investigated by this assay.
Based on these observations the test platform was optimized: here we present a novel test platform for the identification of potential inhibitors of Campylobacter infection based on a ELISA technique. Furthermore with the cell culture model mentioned above, the process of invasion of Campylobacter into chicken cells can be investigated.
[1] The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J. 2011;45:2090.
[2] Jeon B, Muraoka WT, Zhang Q, 2010, Review Advances in Campylobacter biology and implications for biotechnological applications. Microb Biotechnol. 2010 May; 3(3):242-58.
[3] Hermans D, Van Deun K, Messens W, Martel A, Van Immerseel F, Haesebrouck F, Rasschaert G, Heyndrickx M, Pasmans F. 2011. Review Campylobacter control in poultry by current intervention measures ineffective: urgent need for intensified fundamental research. Vet Microbiol. 2011 Sep 28; 152(3-4):219-28.

Board No: 26 *Pathogen-Cell Interaction and Immunity*

EFFECT OF ANTIHELMINTHIC TREATMENT ON VACCINE IMMUNOGENICITY IN PRIMARY SCHOOL CHILDREN IN LAMBARÉNÉ, GABON

S. Brückner[1,2], S. Agnandji[2], S. Berberich[2], B. Abossolo[2], E. Bache[2], J. Francisco Fernandes[2], B. Schweiger[3], M. Massinga Loembe[2], T. Engleitner[1], J. Elias[4], A. Adegnika[1,2], P. Kremsner[1,2], M. Esen[1,2]

[1]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany, [2]Centre De Recherche Médical De Lambaréné, Albert Schweitzer Hospital, Lambaréné, Gabon, [3]Nationales Referenzzentrum Robert Koch Institut, Robert Koch Institute, Berlin, Germany , 4.Institute for Hygiene and Microbiology/ Nrzm, University of Würzburg, Würzburg, Germany

Infection with helminthes is considered as a neglected tropical disease and is a major public health problem especially in the tropics. The influence on cognitive and physical development as well as on the immune system is tremendous. Recent studies showed that individuals infected with helminthes have a reduced antibody response to vaccination. To find out if a single-dose antihelminthic treatment prior to vaccination increases the immune responses to different vaccines, a randomized placebo-controlled double-blind trial in Lambaréné, Gabon from January 2012 to November 2012 was conducted. Three hundred and ten schoolchildren aged 6-10 were enrolled. One group (n=98) received a seasonal influenza vaccine, the second group a meningococcal vaccine (n=106) and the third group (n=106) a cholera vaccine. Four weeks prior to the vaccination children received a single-dose antihelminthic treatment (albendazole 400mg). Vaccine-specific antibodies were assessed at d0 (baseline), d28 and d84. Additionally differences in the development of memory-B-cells represented by antibody secreting cells (ASC) at d0 and d84 were assessed by B-cell ELISpot assay established for each vaccine. We could show that the vaccine-specific titres and ASC increased significantly following the vaccination. Influence of a single-dose antihelminthic treatment was not as efficient as initially assumed.

Board No: 27 *Pathogen-Cell Interaction and Immunity*

PATHOMECHANISMS IN *CAMPYLOBACTER JEJUNI* DIARRHEA

R. Bücker[1], S.M. Krug[1], V. Moos[2], T. Schneider[2], C. Bojarski[2], J. Schulzke[1], C. Barmeyer[1]

[1]Institute of Clinical Physiology, Charité – University Medicine Berlin, Berlin, Germany, [2]Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité – University Medicine Berlin, Berlin, Germany

Introduction: *Campylobacter jejuni* infection causes diarrhea and inflammation. The objective of the present study was to characterize epithelial barrier and ion transport properties in the intestine of *C. jejuni*-infected patients.
Methods: Intestinal biopsies were taken from hospitalized patients during routine endoscopy. In human colon specimens, transepithelial electrical resistance, impedance spectroscopy, electrogenic sodium transport and tracer fluxes were measured in miniaturized Ussing chambers. Tight junctions in affected tissue were analyzed in their protein composition by Western blotting and confocal laser-scanning microscopy and their ultrastructure was studied by freeze-fracture electron microscopy.
Results: Colonic mucosa from *C. jejuni*-infected patients showed an impairment of epithelial barrier function as indicated by a decreased transepithelial electrical resistance (32 ± 8 ohm·cm² versus 57 ± 6 ohm·cm² in healthy controls ($P<0.05$, n=5)). Paracellular permeability to fluorescein (332 Da) increased from 0.2 ± 0.1 · 10-6 cm/s in control to 2.9 ± 0.7 · 10-6 cm/s ($P<0.01$, n=5). Tight junction protein expression was impaired as barrier-forming claudins were down-regulated and re-distributed. Furthermore the epithelial sodium channel ENaC was affected as a reduction of the amiloride-sensitive electrogenic sodium transport from 11 ± 3 µmol/h×cm² in control to 1 ± 1 µmol/h×cm² in *Campylobacter* infection was measured ($P<0.05$).
Conclusions: The pathological changes in *Campylobacter jejuni* diarrhea are characterized by epithelial barrier dysfunction with tight junction dysregulation and sodium malabsorption due to an impairment of the epithelial Na+ channel (ENaC) in the colon. Thus, diarrhea in these patients is due to a leak flux as well to a malabsorptive mechanism.

Board No: 28 *Pathogen-Cell Interaction and Immunity*

FLAVIVIRUS TRANSLOCATION ACROSS THE EPITHELIAL BARRIER – TICK-BORNE ENCEPHALITIS VIRUS TRAFFICKING IN HUMAN INTESTINAL CACO-2 CELL MONOLAYERS

C. Yu[1], K. Achazi[2], L. Möller[3], J. Schulzke[4], M. Niedrig[1], R. Bücker[4]

[1]Centre for Biological Threats and Special Pathogens, Zbs 1: Highly Pathogenic Viruses, Robert Koch Institute, Berlin, Germany, [2]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany, [3]Centre for Biological Threats and Special Pathogens, Zbs 4: Advanced Light and Electron Microscopy, Robert Koch Insitute, Berlin, Germany, [4]Institute of Clinical Physiology, Charité – University Medicine Berlin, Berlin, Germany

Introduction: Tick-borne encephalitis virus (TBEV) is one of the most important vector-borne viruses in Europe and Asia. Its transmission mainly occurs by the bite of an infected tick. However, consuming milk products from infected livestock animals caused TBEV infection, too. To better understand TBEV transmission via the alimentary route, we studied viral infection of human intestinal epithelial cells.
Methods: Caco-2 cells were used to investigate pathological effects of TBEV infection. Cells were grown to confluence on permeable filter supports to form a tight epithelial barrier and were then infected with MOI of 1 or 0.1 from the apical side. Cytological appearance and electrophysiological properties of infected cells were studied.
Results: TBEV-infected Caco-2 monolayers showed morphological changes including cytoskeleton re-arrangements and cytoplasmic vacuolization. Ultrastructural analysis revealed dilatation of the rough endoplasmic reticulum and further enlargement to TBEV containing caverns. Caco-2 monolayers maintained an intact epithelial barrier with stable transepithelial electrical resistance (TER) during the early stage of this infection. Concomitantly, viruses were detected in the basolateral medium, implying a transcytosis pathway. When Caco-2 cells were pre-treated with inhibitors of cellular pathways of endocytosis (EIPA, Cytochalasin, Nocodazole, LY294002), TBEV cell entry was efficiently blocked, suggesting that actin filaments and microtubules are important for PI3K-dependent virus endocytosis. Moreover, experimental fluid uptake assay showed increased intracellular accumulation of FITC-dextran containing vesicles. Immunofluorescence microscopy revealed co-localization of TBEV with early endosome antigen-1 (EEA1) as well as with sorting nexin-5 (SNX5), pointing to macropinocytosis as trafficking mechanism.

In the late phase of the infection, further evidence was found for translocation of virus via the paracellular pathway. Five days after infection TER was slightly decreased. Epithelial barrier integrity was impaired due to increased epithelial apoptosis, leading to passive viral translocation.
Conclusions: Translocation of TBEV across the Caco-2 monolayer was facilitated by macropinocytosis as well as paracellular leakage and apoptosis induction. The findings illuminate pathomechanisms in TBEV infection of human intestinal epithelial cells and viral transmission via the alimentary route.

Board No: 29 *Pathogen-Cell Interaction and Immunity*

DEEP SEQUENCING ANALYSIS OF VIRAL RNA ASSOCIATED WITH PKR DURING INFLUENZA A VIRUS INFECTION

M. Budt[1], F. Jolmes[1], P.W. Dabrowski[2], A. Radonić[2], A. Nitsche[2], T. Wolff[1]

[1]Fg17, Robert Koch Institute, Berlin, Germany, [2]Zbs1, Robert Koch Institute, Berlin, Germany

The protein kinase R (PKR) is a central antiviral effector during influenza A virus (IAV) infection. PKR is activated by binding to double-stranded RNA (dsRNA) or 5'-triphosphorylated stem-loop RNAs. After dimerization and autophosphorylation, it phosphorylates various downstream target molecules, among them the translation initiation factor eIF2α, which leads to a global protein translation block. The non structural protein 1 (NS1) is the main viral PKR inhibitor of influenza virus, yet the mechanism of PKR inhibition is not well understood. In the past, many studies have used artificial ligands like poly-IC or *in vitro*-transcribed RNA to examine PKR activation. However, there is little understanding of the exact nature of the RNA ligands activating PKR in cells infected with influenza. Here, we characterised PKR-bound RNAs isolated from IAV-infected cells. PKR was isolated by immunoprecipitation from infected cells and in a second step, the associated RNA molecules were purified and subjected to deep RNA sequencing on an Illumina HiSeq1500.

A significantly higher amount of PKR-associated RNA was obtained from cells infected with an NS1-deficient (ΔNS1) compared to wt-virus infected cells. With both viruses, a preferential association of PKR with the long viral gene segments, encoding the polymerase subunits was observed. Strikingly, however, only in wt-, but not in ΔNS1-infected cells, we observed a predominant mapping of reads near the ends of the polymerase segments. This finding indicates a preferential binding of PKR to subgenomic defective-interfering (DI) RNA in the presence of NS1, although the similar presence of DI-RNA in both wt-and ΔNS1-infected cells was verified.

In ΔNS1-infected cells, PKR showed even stronger binding to polymerase segment-derived RNA, but the reads were equally distributed over the entire segments, indicating a preference for full-length genomic RNA. Notably, only PKR-associated RNA from ΔNS1- but not wt- infected cells could stimulate PKR after retransfection, indicating that the presence of NS1 prevents access of PKR to stimulating RNA species. We conclude, that NS1 affects both the selectivity as well as the efficiency of PKR binding to viral RNA to prevent its activation. These findings reveal new twists in the regulation of a central antiviral effector.

Board No: 30 *Pathogen-Cell Interaction and Immunity*

MASS SPECTROMETRY-BASED PROTEIN EXPRESSION ANALYSIS FOR THE ELUCIDATION OF PATHOGENIC MECHANISMS OF COWPOX VIRUSES

M. Bürger, J. Döllinger, A. Nitsche

Centre for Biological Threats and Special Pathogens: Highly Pathogenic Viruses (Zbs 1), Robert Koch Institute, Berlin, Germany

Cowpox viruses (CPXV) are complex dsDNA viruses coding for about 200 proteins. The pathogenic as well as zoonotic potential may vary from strain to strain but the molecular mechanisms are hardly known so far.
To elucidate the mechanisms underlying the varying virulence of CPXV, comparative protein expression analysis of a reference CPXV strain Brighton Red (BR) and a higher virulent zoonotic wild-type CPXV strain isolated from a rat (WT) were carried out. Human keratinocytes (HaCaT) were used as a model close to the regular entry route of CPXV infections and were infected with MOI = 5. Cytoplasmic and secreted proteins were quantified with nLC-ESI MS/MS (LTQ Orbitrap) 2, 6 and 10 h p.i. using Stable Isotope Dimethyl Labeling. Functional annotation of host cell proteins was carried out with Cytoscape GeneMANIA. Furthermore, the amount of released virus particles in the supernatant as well as cell status during the time course of infection of different cell lines were observed using plaque assay and xCELLigence system.
It could be shown that CPXV WT infected cells release higher amounts of infectious particles in contrast to CPXV BR leading to faster cell death. Furthermore, non-modified as well as differently regulated host cell proteins reflecting various cellular processes during CPXV BR and CPXV WT infection could be identified.
From this study it can be hypothesized that differences in the virulence of both CPXV strains may result from a differently regulated host immune response during infection with both CPXV strains.

Board No: 31 *Pathogen-Cell Interaction and Immunity*

MASS SPECTROMETRIC ANALYSIS OF RIG-I REVEALS A POTENTIAL ROLE OF DDX6 IN INFLUENZA VIRUS INFECTION

R. Daviña Nuñez, M. Budt, S. Sänger, A. Sadewasser, K. Paki, T. Wolff

Division of Influenza and Other Respiratory Viruses, Robert Koch Institute, Berlin, Germany

Influenza virus infection is detected by the cellular pattern recognition receptor (PRR) retinoic acid inducible gene-I (RIG-I) receptor. (RIG-I)-like receptors recognize cytoplasmic viral RNA and induce the production of type I interferons (IFNs) and proinflammatory cytokines. To gain a better understanding of the role of RIG-I during influenza virus infection, we employed Stable-Isotopic-Labeling-of-amino-acids-in-Cell-Culture (SILAC), a mass spectrometric technique that allows relative protein quantification, to identify novel RIG-I interaction partners. Within the proteins that displayed an increased binding to RIG-I we found an enrichment of RNA binding proteins, among them, the RNA helicase DDX6.
DDX6 is an RNA helicase involved in mRNA metabolism and it is a component of P-bodies and stress granules. We confirmed the association between RIG-I and DDX6 by coimmunoprecipitation/immunoblot analysis of the endogenous proteins in infected and non-infected cells. Further we analyzed RIG-I and DDX6 distribution by confocal microscopy. DDX6 was localized in P-bodies, as expected, and presented an additional diffuse staining in the cytoplasm, that was similarly observed for RIG-I. In cells infected with an engineered influenza B virus DDX6 colocalized with RIG-I in stress granules. Interestingly transfection based reporter assays revealed that DDX6 overexpression enhances RIG-I mediate interferon ß promotor activation. These results were further confirmed by showing increased production of IFNß transcripts in infected cells upon DDX6 overexpression.
DDX6 is an RNA helicase involved in mRNA metabolism. Here we show that DDX6 is a novel RIG-I regulator that enhances its activation, suggesting a role for DDX6 in innate immunity.

Board No: 32 *Pathogen-Cell Interaction and Immunity*

COMPARISON OF TWO NEUTROPHIL EXTRACELLULAR TRAP (NET) EVASION FACTORS IN *STREPTOCOCCUS SUIS*

N. de Buhr[1], A. Neumann[2], P. Valentin-Weigand[1],M. von Köckritz-Blickwede[2], C.G. Baums[3]

[1]Institute for Microbiology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Hannover, Germany, [2]Department of Physiological Chemistry, University of Veterinary Medicine Hannover, Hannover, Germany, [3]Institute for Bacteriology and Mycology, Centre for Infectious Diseases, College of Veterinary Medicine University Leipzig, Leipzig, Germany

Streptococcus (S.) suis infections can lead to a suppurative meningitis in pigs and humans, characterized by infiltration of a high number of neutrophil granulocytes. A recently discovered defence mechanism of the innate immune system is the formation of neutrophil extracellular traps (NETs). Bacterial extracellular DNases are described as a main NET evasion mechanism of various bacteria. In this study we investigated the interaction of *S. suis* with NETs and the role of two DNases expressed by *S. suis* under different conditions, SsnA and DNaseSuisII. For this, deletion mutants of both nucleases as well as a double mutant were compared phenotypically to the wild-type strain. Firstly, growth in standard growth medium, human cerebrospinal fluid and blood was investigated. Furthermore, the DNase activity at different growth phases was tested. Secondly, the degradation of NETs in presence of the wild-type strain and both mutants was analyzed *in vitro* by immunofluorescence microscopy, and survival of the strains in the presence of NETs was analysed. Thirdly, the transcription levels of both DNases were investigated at exponential and stationary growth phase by qRT-PCR.

No significant differences in growth between *S. suis* wild-type and the nuclease mutants in Todd-Hewitt broth, cerebrospinal fluid and blood were detected. Depending on the experimental conditions and the growth phase an attenuation in DNase activity was found. Interestingly, we were able to show that both nucleases contribute to NET degradation, though their highest activity was detected at different growth phases. Our ongoing studies are aimed to reveal the specific role and synergisms of both nucleases in host-pathogen interaction.

Board No: 33 *Pathogen-Cell Interaction and Immunity*

VIRUS SUPPORTIVE FUNCTION OF THE MACROAUTOPHAGY-RELATED PROTEINS BECLIN1 AND ATG7 DURING INFLUENZA A VIRUS INFECTION

S.E. Dudek, S. Ludwig

Institute of Molecular Virology, Center for Molecular Biology of Inflammation, Westfälische Wilhelms University of Münster, Münster, Germany

Macroautophagy is a cellular process, which directs cytoplasmic cellular components as well as invading pathogens to lysosomes for lysosomal degradation. On the induction of macroautophagy a complex interplay of different autophagy-related genes/ proteins (Atgs) takes place. For many viruses interplay with macroautophagy is documented, which may have virus-supportive as well as antiviral features.
Influenza A viruses (IAV) are well known to interact with many different proviral and antiviral cellular signaling pathways to ensure their replication. Recent publications report interplay of IAV with the macroautophagy machinery; however, a detailed picture of the influence on viral replication is still missing. Here we analyzed the impact of different autophagy-related genes (Atgs) on IAV life cycle. While a knock-down of Atg7 and Beclin1 leads to reduced viral titers in A549 cells, no differences were obtained when Atg5 or Atg12 were missing. Further, the knock-down of Atg7 and Beclin1 reduced IAV-induced type I IFN signaling in a stronger way compared to a lack of Atg5 and Atg12. However, we excluded a major role of type I IFN response in reducing viral titers since titers were also decreased in IFN-deficient Vero cells treated with Atg7 and Beclin1 siRNA. Further investigations revealed that the synthesis of viral mRNAs was not affected by the lack of Atg7 and Beclin1. Nevertheless, reduced viral protein accumulation was observed when Atg7 and Beclin1 were missing in A549 as well as Vero cells. We conclude that Atg7 and Beclin1 are functioning to support efficient IAV replication by interfering with viral protein accumulation.

Board No: 34 *Pathogen-Cell Interaction and Immunity*

NOVEL ANTI-INFECTIVE STRATEGIES AGAINST INFLUENZA A VIRUS AND S. AUREUS INFECTIONS

C. Ehrhardt[1], A. van Krüchten[1], A. Christersson-Wiegers[1], O. Planz[2], S. Ludwig[1]

[1]Westfälische Wilhelms University, Institute of Molecular Virology, Münster, Germany, [2]Department of Immunology, University of Tübingen, Interfaculty Institute for Cell Biology, Tübingen, Germany

Infections with influenza A viruses (IAV) are still amongst the major causes of highly contagious severe respiratory diseases, not only bearing a devastating burden to human health, but also significantly affecting the economy. Another problem concerns increased fatality rates, linked to secondary bacterial pneumonia, caused by pathogens such as *Staphylococcus aureus* (*S. aureus*). Besides vaccination that represents the best option to get protected from IAV infections, only two classes of anti-influenza drugs, inhibitors of the viral M2 ion channel and the viral neuraminidase, have been approved. Furthermore, seasonal and pandemic IAV show a rapid development of resistant variants against the currently licensed therapeutics. Similarly, for antibacterial intervention highly effective antibiotics are available, but there is a frightening increase in resistant strains. Thus, an urgent need for novel anti-infective strategies targeting both pathogens is obvious.
In different studies we have identified virus-supportive cellular functions as potential targets for antiviral intervention. Among these, the cellular IKK/NF-kappaB signalling pathway was shown to regulate the viral ribonucleoprotein export out of the nucleus. Inhibition of NF-kappaB signalling results in reduced expression of cytokines, chemokines, and pro-apoptotic factors and subsequent inhibition of caspase activation and block of caspase-mediated nuclear export of viral ribonucleoproteins. In consequence, the production of progeny viruses is reduced. Here we demonstrate that targeting NF-kappaB signalling by usage of the SC75741 inhibitor or the acetylsalicylic acid based compound LASAG inhibits IAV replication with reduced tendency to induce resistant virus variants. Furthermore, we provide first evidence that inhibition of NF-kappaB signalling also affects the load of intracellular bacteria in an *in vitro* model-system independent of the presence and absence of IAV. We will discuss the usability of chemical compounds targeting cellular factors as therapeutic agents during infections with influenza viruses and *S. aureus*.

Board No: 35 *Pathogen-Cell Interaction and Immunity*

ECTODOMAIN SHEDDING OF THE TUPAIA PARAMYXOVIRUS F PROTEIN

T. Enkirch[1], F. Wirth[1], B. Hoyler[2], C. Springfeld[3], V. von Messling[1]

[1]Division of Veterinary Medicine, Paul Ehrlich Institut, Langen, Germany, [2]Department of Internal Medicine, Heidelberg University Hospital, Heidelberg, Germany, [3]Department of Medical Oncology, National Center for Tumor Diseases, Heidelberg, Germany

The *Tupaia paramyxovirus* (TPMV) was first isolated from kidney cells of an apparently healthy tree shrew (*Tupaia belangeri*). Analysis of the nucleotide sequence of the N and P gene revealed typical characteristics of the subfamily *Paramyxovriринae* and provided evidence for a close phylogenetic relationship to the *Henipaviridae* and *Morbilliviridae*. As for the other paramyxoviruses, the TPMV F protein mediates virus-cell and cell-cell fusion. It is expressed as a F0 precursor protein and processed during maturation into F1 and F2 fragments linked by a disulfide-bond. To characterize TPMV F protein processing in more detail, an antiserum against a peptide in the ectodomain of the F1 fragment (αFecto) was generated. This led to the detection of an additional fragment with a molecular mass of about 45 kDa, suggesting that part of the F proteins undergo an additional cleavage, resulting in the fragments F1a, F1b and F2. Investigation of the glycosylation pattern of that new fragment revealed that in contrast to F0, the F1 and F1a fragments were almost completely resistant towards endoH treatment, demonstrating that the F0 fragment was mainly located intracellular whereas the F1 and also the F1a fragment were transported to the cell surface. The F1a fragment was also detected in the supernatant of transfected 293T cells, suggesting that it is secreted. Generation of truncated F proteins revealed that cleavage occurs between amino acids 499 and 500, which are located in the predicted transmembrane domain. A similar F1a/F1b cleavage was already observed for members of the Morbilli- and Rubulavirus genus, indicating that it plays an important role in the life cycle of several members of the *Paramyxoviridae* family.

Board No: 36 *Pathogen-Cell Interaction and Immunity*

APPROVED DRUGS WITH BROAD ANTI-INFLUENZA A ACTIVITY

T. Enkirch[1], S. Sauber[1], E. Gan[2], S. Maurer-Stroh[3], V. von Messling[1,2]

[1]Division of Veterinary Medicine, Paul Ehrlich Institut, Langen, Germany, [2]Emerging Infectious Disease Program, Duke Nus Graduate Medical School, Singapore, Singapore, [3]Bioinformatics Institute, Agency for Science, Technology and Research, Singapore, Singapore

Influenza A virus is one of the most important causes of respiratory infections worldwide. In addition to annual epidemics, the emergence of new antigenic variants can result in pandemics with increased morbidity and mortality. Because of its broad host range which includes many avian and mammalian species, an eradication in not feasible. Since resistance mutations rapidly emerge for drugs targeting viral proteins, this project aimed at assessing the antiviral activity of already approved drugs known to target cellular proteins that are involved in the influenza virus life cycle. The drugs were initially screened for their ability to inhibit the replication of the mouse-adapted influenza strain A/Puerto Rico/8/34 in Madin-Darby canine kidney cells. The proportion of infected cells after treatment was then quantified by flow cytometry to determine the therapeutic window. Out of 15 compounds, four were able to inhibit infection ten to hundred fold without causing toxicity *in vitro*. Three of the drugs resulted in 80 % reduction in infected cells. Candidates that reduced infection at least tenfold were further evaluated *in vivo*. Towards this, mice were treated orally starting one day before infection and continuing for four days. Three days post infection, the animals were sacrificed and the viral load in the lung was compared to untreated controls. Only two drugs resulted in a significant reduction of lung titers, displaying an efficacy profile similar to the results obtained with the approved influenza inhibitor amantadine. We are currently assessing the inhibitory activity of these compounds against seasonal and highly pathogenic influenza viruses and are planning to evaluate the efficacy of the most promising candidates against different strains in ferrets. Here we show that drugs targeting cellular proteins instead of viral proteins are a promising approach for influenza treatment. This proof-of-concept study is a first step towards the use of these drugs to mitigate influenza disease and supports further evaluation in other pre-clinical models and possibly clinical trials.

Board No: 37 *Pathogen-Cell Interaction and Immunity*

THE DISTINCT ROLES OF MATRIXMETALLOPROTEINASES-2 AND -9 IN MEDIATING MURINE *CAMPYLOBACTER JEJUNI* INDUCED ENTEROCOLITIS

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

[1]Department for Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany, [2]Department of Medicine I for Gastroenterology, Infectious Disease and Rheumatology / Research Center ImmunoSciences (RCIS), Charité University Medicine Berlin, Berlin, Germany

Background: Matrixmetalloproteinases (MMPs) comprize a tighly controlled heterogenous family of matrix-degrading endopeptidases which are physiologically involved in tissue development, differentiation, proliferation and regeneration. A dysbalance between activators and inhibitors of MMP expression, however, results in diseases such as arthritis, atherosclerosis, or cancer. Furthermore, the gelatinases A and B (MMP-2- and MMP-9, respectively) are upregulated in human inflammatory bowel diseases. In this study we for the first time investigated the impact of gelatinases A and B in experimental *Campylobacter (C.) jejuni* induced enteritis.

Methodology/Principal Findings: Given that conventional mice display a physiological colonization resistance against *C. jejuni* due to their intestinal microbiota composition, we generated gnotobiotic MMP-2-/- and MMP-9-/- mice following broad-spectrum antibiotic treatment to assure stable colonization upon peroral infection with *C. jejuni* strain 81-176. C. jejuni colonized the intestines of gnotobiotic mice irrespective of the genotype at comparable loads. *C. jejuni* infected MMP-2-/-, but not MMP-9-/- mice displayed lower colonic apoptotic cell numbers at day 14 post infection (p.i.). Both, MMP-2-/- and MMP-9-/- infected mice, however, exhibited less distinct pro-inflammatory immune responses in the colon as compared to wildtype controls as indicated by lower numbers of colonic T lymphocytes, macrophages and neutrophils and concomitant lower IL-1beta and TNF-alpha mRNA expression levels. Furthermore, gnotobiotic IL-10-/- mice suffering from severe *C. jejuni*-induced enterocolitis within one week p.i. were perorally treated with the selective gelatinase inhibitor RO28-2653 from d1 until d6 p.i. (75 mg/kg body weight/day, once daily). Remarkably, RO28-2653 treatment of *C. jejuni* infected gnotobiotic IL-10-/- mice ameliorated ulcerative enterocolitis as indicated by significantly less clinical pathology (i.e. bloody diarrhea), lower numbers of Casp3+ apoptotic cells,

but higher number of Ki67+ proliferating cells in the colonic mucosa at day 7 p.i. as compared to placebo control mice. Furthermore RO28-2653 treatment resulted in less distinct influx of T and B cells as well as macrophages into the colonic mucosa of *C. jejuni* infected mice as compared to placebo treated controls. Taken together, MMP-2 and MMP-9 are both essentially involved in *C. jejuni* induced immunopathology.

Conclusion/Significance: Selective gelatinase blockage might be a promising treatment option of (e.g. *C. jejuni* induced) intestinal inflammation in humans.

Board No: 38 *Pathogen-Cell Interaction and Immunity*

NUCLEOTIDE-OLIGOMERIZATION-DOMAIN-2 AFFECTS COMMENSAL GUT MICROBIOTA COMPOSITION AND INTRACEREBRAL IMMUNOPATHOLOGY IN ACUTE *TOXOPLASMA GONDII* INDUCED MURINE ILEITIS

M.M. Heimesaat[1], I.R. Dunay[2], M. Alutis[1], A. Fischer[1], L. Möhle[2], U.B. Göbel[1], A.A. Kühl[3], S. Bereswill[1]

[1]Department for Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany, [2]Department for Microbiology and Hygiene, University of Magdeburg, Magdeburg, Germany, [3]Department of Medicine I for Gastroenterology, Infectious Disease and Rheumatology / Research Center Immuno¬sciences (RCIS), Charité University Medicine Berlin, Berlin, Germany

Background: Within one week following peroral high dose infection with *Toxoplasma (T.) gondii*, susceptible mice develop non-selflimiting acute ileitis due to an underlying Th1-type immunopathology. The role of the innate immune receptor nucleotide-oligomerization-domain-2 (NOD2) in mediating potential extra-intestinal inflammatory sequelae including the brain, however, has not been investigated so far.
Methodology/Principal Findings: Following peroral infection with 100 cysts of *T. gondii* strain ME49, NOD2-/- mice displayed more severe ileitis and higher small intestinal parasitic loads as compared to wildtype (WT) mice. However, systemic (i.e. splenic) levels of pro-inflammatory cytokines such as TNF and IFN-gamma were lower in NOD2-/- mice versus WT controls at day 7 p.i. Given that the immunopathological outcome might be influenced by the intestinal microbiota composition, which is shaped by NOD2, we performed a quantitative survey of main intestinal bacterial groups by 16SrRNA analysis. Interestingly, Bifidobacteria were virtually absent in NOD2-/- but not WT mice, whereas differences in remaining bacterial species were rather subtle. Interestingly, more distinct intestinal inflammation was accompanied by higher bacterial translocation rates to extra-intestinal tissue sites such as liver, spleen, and kidneys in *T. gondii* infected NOD2-/- mice. Strikingly, intracerebral inflammatory foci could be observed as early as seven days following *T. gondii* infection irrespective of the genotype of animals, whereas NOD2-/- mice exhibited higher intracerebral parasitic loads, higher F4/80 positive macrophage and microglia numbers as well as higher IFN-gamma mRNA expression levels as compared to WT control animals.

Conclusion/Significance: NOD2 signaling is involved in protection of mice from *T. gondii* induced acute ileitis. The parasite-induced Th1-type immunopathology at intestinal as well as extra-intestinal sites including the brain is modulated in a NOD2-dependent manner.

Board No: 39 *Pathogen-Cell Interaction and Immunity*

IDENTIFICATION OF EPITOPES OF THE 47-KDA AND 56-KDA OUTER-MEMBRANE PROTEINS OF *ORIENTIA TSUTSUGAMUSHI*, THE AGENT OF SCRUB TYPHUS

H.J. Jeong[1], S. Choi[1], B. Gill[1], Y. Ju[2], Y. Seon Lee[1], J. Lee[1], K. Hwang[1]

[1]Division of Zoonoses, Korea Centers for Disease Control and Prevention, Chungcheongbuk Do, Republic of Korea, [2]Division of Medical Entomology, Korea Centers for Disease Control and Prevention, Chungcheongbuk Do, Republic of Korea

Scrub typhus, caused by *Orientia tsutsugamushi*, is endemic to the countries in the Asia-Pacific region, including Korea, Japan, China, Russia, Southeast Asian countries, and northern Australia. It is a zoonotic disease transmitted by the bite of trombiculid mite larvae in endemic rural areas. The clinical features of the disease are fever, rash, eschar, pneumonia, and myocarditis. Two major outer-membrane proteins (OMPs) of 47 kDa and 56 kDa have been considered potential candidates for vaccines and/or diagnostic antigens. In this study, we identified several epitope-clusters by epitope-scanning analysis of these proteins of the *O. tsutsugamushi* Boryong strain prevalent in Korea. We found that amino acids 41-59, 177-208, 361-383, and 421-436 in the 47-kDa OMP and amino acids 49-99, 153-239, and 268-323 in the 56-kDa OMP were the most reactive to antisera from scrub typhus patients. Using synthetic peptides based on these protein epitopes, we observed individual patient-dependent patterns of serological reactivities with the 56-kDa OMP-based peptides but overall reactivities with the 47-kDa OMP-based peptides were low. The reactivities with the peptides to antisera from mice immunized with other serotypes were also compared. These results will be useful for designing more reactive antigen(s) with a broad host-range for the development of vaccines and/or serological diagnostic tools for scrub typhus.

Board No: 40 *Pathogen-Cell Interaction and Immunity*

THE AVIAN ORTHOLOGUE OF CCL16 PLAYS AN IMPORTANT ROLE IN SALMONELLA INFECTION

M. Reger[1], S. Härtle[1], A. Berndt[2], U. Methner[2], L. Wieler[3], V. Waters[4], P. Kaiser[4], B. Schusser[1], B. Kaspers[5]

[1]Department for Veterinary Sciences, University of Munich, Munich, Germany, [2]Federal Research Institute for Animal Health, Friedrich Loeffler Institute, Jena, Germany, [3]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany, [4]The Roslin Institute, University of Edinburgh, Ester Bush, United Kingdom, [5]University of Munich, Institute for Animal Physiology, München, Germany

Food-borne zoonotic infections of humans are still a major problem in Europe. Over 99000 people were infected with Salmonella in the year 2010 (EFSA), some with fatal outcome. The control of infections in birds, which are asymptomatic carriers, is an important strategy to prevent human infections. Therefore, a comprehensive understanding of the immune response of chicken is crucial to develop new control strategies. In order to better understand the innate response of birds to infection, we performed global gene expression analysis using the micro-array technology, which led to the identification of candidate genes supposed to play an important role in the early immune defence of chicken against *Salmonella enterica* (S.e.) infections.

The CC-chemokine K203, which is supposed to be an ortholog of human CCL16, is amongst the most strongly induced genes in Salmonella infected primary macrophages and gut tissue. More detailed gene expression analysis of activated primary chicken macrophage cultures revealed that K203 is induced by a broad range of pathogen associated molecular patterns. In addition, K203 mRNA is rapidly induced upon Salmonella infection in caecal tissue and remains strongly elevated for at least 14 days p.i. suggesting an important role in immune cell attraction and pathogen clearance. In order to identify K203 responsive cells, we cloned and expressed the chemokine as a Fc-tagged version and performed binding studies with blood and spleen derived leucocytes. 100% of blood monocytes and subpopulations of CD4+ and CD8+ T-lymphocytes bound K203-Fc in a dose dependent manner. Putative receptors were identified by chicken genome analysis and expressed in HEK 293 cells as GFP-tagged proteins. CCRa and CCRb but not CCRc bound K203-Fc and were thus identified as the true receptors which are coexpressed on monocytes and CD4+ and CD8+ T-cell subsets.

Transwell migration assays confirmed that K203 is a chemo-attractant for monocytes. Immunhistological studies of *S.e.* infected caeca showed that within 2 days after infection large numbers of monocytes/macrophages accumulate in the lamina propria synchronized with K203 expression. Based on these studies we propose a model in which Salmonella infection triggers K203 production by resident macrophages thus attracting large numbers of monocytes into the infected tissue to support the elimination of the pathogen.

This work was supported by the BMBF FBI-Zoo program.

Board No: 41 *(New and Re-) Emerging Zoonotic Diseases*

TOXOPLASMA GONDII INFECTION IN PATIENTS WITH SCHIZOPHRENIA

H.M. Bakre[1,2], S.K. Hussain[2], S.K. Ali[3]

[1]Department of Veterinary Microbiology and Immunology, Hawler Medical University, Iraq/erbil, Iraq, [2]Institute of Medical Microbiology, University, Erbil, Iraq, [3]Psychiatry, University, Erbil, Iraq

Keywords: Toxoplasmosis, Toxoplasma gondii, Schizophrenia, C-reactive protein

Background: Schizophrenia is a complex chronic neuropsychiatric disease of the central nervous system, believed to have multiple etiologies. *Toxoplasma gondii* has emerged as an interesting candidate as a possible cause of some cases of schizophrenia. As there is scarce information about the seroprevalence of *T.gondii* infection in psychiatric patients in Erbil; we investigated the seroprevalence of *T.gondii* in schizophrenic patients and compared with that obtained from control individuals in Erbil correlated with inflammatory marker C-reactive protein (CRP).
Method: This case control study included ninety three (93) schizophrenic patients seeking medical advice at Hawler psychiatric hospital and private clinics with ninety three (93) non psychiatric control were screened for the presence of anti-toxoplasma IgG, IgM (by ELISA test) and C-reactive protein(CRP) using qualitative methods. Questionnaires were used to collect socio-demographic and behavioral data among the respondents.
Results: In chronic cases anti-*Toxoplasma gondii* IgG antibodies were seropositive in 30/93 (32.3%) of the schizophrenic patients and 4/93(4.3%) of control (P< 0.001). The seropositive rate of IgM antibodies was 9.7% and 1.1% among schizophrenic patients and control respectively (P=0.006). The result of C-reactive protein (CRP) positivity among patients and control was 23.6% and 3.22% respectively (p<0.001).
Conclusion: Our results delineate that association might be exist between *Toxoplasma gondii* infection and schizophrenia etiology

Board No: 42 *(New and Re-) Emerging Zoonotic Diseases*

A NOVEL ELISA ASSAY BASED ON HENDRA VIRUS N PROTEIN EQUALLY DETECTS NIPAH AND HENDRA VIRUS ANTIBODIES IN SERA OF DIFFERENT SPECIES

L. Kwasnitschka[1], K. Fischer[1], M.H. Groschup[1], S. Finke[2], G.M. Keil[2], A. Balkema-Buschmann[1]

[1]INEID, Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany, [2]IMVZ, Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany

Hendra and Nipah viruses may cause severe respiratory and encephalitic syndromes predominantly in horses (Hendra virus) and pigs (Nipah virus) in Southeast Asia. Both viruses, which comprise a new genus designated Henipavirus in the subfamily Paramyxovirinae, can cause deadly infections in humans after direct contact to infected animals or their secretions, and have therefore been classified as BSL4 agents. In order to improve the preparedness for a possible introduction of infected animals into Europe, we have developed an indirect ELISA for antibody detection based on baculovirus-expressed Hendra virus N protein. Using this assay, we were not only able to correctly identify sera from Hendra virus infected horses from a set of positive and negative horses, but also sera from pigs experimentally challenged with Nipah or Hendra virus from a set of positive and negative pigs. In the next step, this assay will be validated on larger sets of positive and negative serum samples from horses and pigs and possibly also from different domestic and wildlife animal species.

Board No: 43 *(New and Re-) Emerging Zoonotic Diseases*

ANALYSIS OF POTENTIAL VIRULENCE FACTORS OF VIBRIO PARAHAEMOLYTICUS ISOLATED FROM GERMAN COASTAL WATERS

S. Bechlars, R. Dieckmann, E. Strauch

Department for Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

Vibrio parahaemolyticus is a recognized enteropathogen causing diarrhea in humans and is a major cause of diarrheal diseases in parts of the world where seafood strongly contributes to nutrition of the population. Transmission occurs mainly through raw or undercooked seafood.
The toxins TDH (thermostable direct hemolysin) and TRH (TDH-related hemolysin) as well as the type III secretion system 2 are considered as major virulence factors since they are strongly correlated with clinical strains. As *tdh* positive strains are not detected in coastal waters of Germany, we focused on the analysis of the pathogenic potential of indigenous *trh* positive strains, isolated from mussels, seawater and a patient.
12 *V. parahaemolyticus* isolates from Baltic and North Sea and 18 strains from other regions and sources were examined by multiplex PCR based virulotyping for detection of potentially virulence-associated targets and phenotypic analyses to assess serum resistance and hemolytic activities. Phylogenetic relationships of the strains were evaluated using multilocus sequence typing (MLST). *Trh* positive strains showed a genetic linkage to *ure* gene cluster (urease) and possessed *t3ss2β*. Sequencing of *trh* genes revealed the presence of 9 variants among the tested strains. All strains could be classified as resistant towards human serum and showed different hemolytic activities depending on the *trh* variant. Furthermore, TRH and VopC expression was analysed under different growth conditions on transcriptional level via qRT-PCR showing a complex expression pattern.
Results of our study suggest that the major virulence markers *trh* and *tdh* might not be sufficient for detection of potentially pathogenic *V. parahaemolyticus* strains. A further differentiation of *trh*variants and the assessment of T3SS2 components could lead to a refinement of the risk assessment in food analyses and clinical diagnostics.

Board No: 44 *(New and Re-) Emerging Zoonotic Diseases*

HUMAN LEPTOSPIROSIS IN MALAYSIA 2004-2012: AN EMERGING ZOONOTIC DISEASE

D. Benacer[1], K. Lin Thong[1], N. Choung Min[2], K. Verasahib[3], S. Nursheena Mohd Zain[1]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia, [2]Institute of Mathematical Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia, [3]Disease Control Division, Ministry of Health, Putrajaya, Malaysia

Leptospirosis is an endemic disease in Malaysia. A dramatic increase of the number of leptospirosis cases has been reported in the last decade. Hence there is a lack of information about this disease in this country. The objective of this current study is to describe the epidemiology of human leptospirosis in Malaysia during 2004–2012.

The data provided by the Disease Control Division, Ministry of Health Malaysia and collected from all hospitals and private health care facilities in the 14 states was analysed according to age, sex, ethic, seasonality and geographic distribution.

A total of 12,325 reported cases of leptospirosis with male subjects accounting for 9696 (78.7%) cases compared to female patients with 2629 (21.3%) cases (p-value < 0.01). The overall ration male: female was 3.50:1. There were 338 death cases with the average annual case-fatality rate was 3.6%. According to the population data based on age groups in 2012 in all the states, the highest incidence occurs in the age group of 30 to 39 years old subjects (16.2) while the lowest incidence occurs in the age group of 0 to 9 years old subjects (3.4). The disease was most common amongst the Malays (60.7%), the largest ethnic group in Malaysia. A positive correlation was found between number of cases and the number of rain days per month and the monthly average temperature (p-value < 0.05).

Leptospirosis is an emergence disease in Malaysia, and it is considered a significant cause of morbidity and mortality. Therefore, continues surveillance of this disease is necessary to understand the epidemiology of this zoonotic illness.

Board No: 45 *(New and Re-) Emerging Zoonotic Diseases*

VIRULENCE CHARACTERISTICS AND ANTIBIOTIC RESISTANCE PATTERNS OF VIBRIO VULNIFICUS ISOLATES FROM THE NORTH AND BALTIC SEA

N. Bier[1], S. Diescher[1], C. Jaeckel[1], R. Dieckmann[1], S. Böer[2],N. Brennholt[2], E. Strauch[1]

[1]Federal Institute for Risk Assessment, Berlin, Germany
[2]Federal Institute of Hydrology, Koblenz, Germany

Vibrio vulnificus is a Gram-negative bacterium that is found in coastal waters worldwide and responsible for severe wound or foodborne infections with high mortality rates. Because of its high multiplication rate in warm waters with moderate salinity concerns have aroused that the presence of this pathogen will increase at the coasts of Northern Europe due to impacts of the climate change. Since 2006, in fact, *V. vulnificus* has repeatedly been detected in many investigated bathing locations in Mecklenburg-Western Pomerania, Germany. Moreover serious cases of wound infections have been reported in the Baltic Sea region, especially in years with hot summers and periods of elevated sea water temperatures. However, despite the frequent occurrence of this pathogen, the number of reported clinical cases is relatively low indicating that not all strains of *V. vulnificus* have the same virulence potential.

In a recent study (Bier *et al.*, 2013), we found that every clinical biotype 1 isolate from the Baltic Sea region possessed at least one of six different virulence characteristics. We therefore proposed to apply a combination of selected methods to assess the pathogenic potential of *V. vulnificus* isolates from this region. In order to estimate the health risk emanating from this pathogen in German coastal waters, 111 *V. vulnificus* isolates mainly from waters and sediments of the North and Baltic Sea, including isolates from designated bathing locations were characterized with the suggested methods: Molecular characterization of polymorphic loci (16S rRNA and virulence correlated gene (vcg), detection of virulence associated genes (pathogenicity region XII and *nan*A gene), as well as phenotypic characterization (mannitol fermentation and resistance to human serum) were performed. The genetic diversity of the isolates was evaluated by multilocus sequence typing (MLST) and the isolates were further examined for antibiotic resistance patterns.

Board No: 46 *(New and Re-) Emerging Zoonotic Diseases*

IMPORTANCE OF ENDOPHILIC TICK *IXODES TRIANGULICEPS* IN THE TRANSMISSION OF *ANAPLASMA PHAGOCYTOPHILUM, CANDIDATUS NEOEHRLICHIA MIKURENSIS* AND *BABESIA MICROTI* IN RODENTS IN SLOVAKIA (CENTRAL EUROPE)

L. Blaňárová[1], M. Stanko[1,2], G. Carpi[3], D. Miklisová[1], B. Víchová[1], L. Mošanský[1], M. Bona[4], J. Kraljik[1,5], B. Peťko[1], M. Derdáková[1,2]

[1]Department of Vector Borne Diseases, Institute of Parasitology Sav, Košice, Slovakia, [2]Department of Medical Zoology, Institute of Zoology of the Sas, Bratislava, Slovakia, [3]Biodiversity and Molecular Ecology Department, Research and Innovation Centre Fondazione Edmund Mach, Trento, Italy, [4]Department of Anatomy, Faculty of Medicine, Upjš, Košice, Slovakia, [5]Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Rodents are important reservoir hosts of tick-borne pathogens. *Anaplasma phagocytophilum* is the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. Presence of human pathogens *Candidatus* Neoehrlichia mikurensis and intraerythrocytic protozoan parasite, *Babesia microti* in *Ixodes ricinus* ticks in Slovakia have been recently proposed. The aim of the work was to identify the prevalence and genetic diversity of pathogens circulating in the natural foci between the rodents and ticks as well as to study their ecological associations. *A. phagocytophilum* was detected in questing *I. ricinus* ticks from all studied sites and in host feeding *I. trianguliceps* ticks, as well as in rodent biopsies. *A. phagocytophilum* was not detected in rodents in areas where *I. trianguliceps* ticks were absent. Moreover, phylogenetic analyses have shown the presence of two distinct clades of *A. phagocytophilum*. The first clade contained *A. phagocytophilum* genotypes from questing *I. ricinus* and feeding *I. ricinus* from a broad array of hosts. The second clade comprised solely genotypes found in rodents and feeding *I. trianguliceps*.*N. mikurensis* was detected in questing *I. ricinus* ticks, spleens of rodents and feeding *I. ricinus* and *I. trianguliceps* ticks from rodents. The 16S rRNA and *gltA* sequences of *N. mikurensis*obtained in this study confirmed high degree of similarity. DNA of *B. microti* was found in biopsies of rodents and feeding and questing *I. ricinus* ticks. None of the 112 *I. trianguliceps* ticks were infected with *B. microti*. Embryos of rodents were also positive for tick-borne pathogens.

In this study we have confirmed that *A. phagocytophilum* strains display specific host and vector associations also in Central Europe similarly to the situation in United Kingdom and that *A. phagocytophilum* genotypes associated with rodents are probably transmitted solely by *I. trianguliceps*ticks. Results of our study also confirmed the importance of rodents in the circulation of *N. mikurensis* and *B. microti* in the natural foci of Slovakia.

The study was supported by the projects of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0.1), VEGA 2/0055/11, VEGA 2/0113/12, VEGA 2/0060/14, APVV – 0267 - 10, EU project FP7 EDENext.

Board No: 47 *(New and Re-) Emerging Zoonotic Diseases*

MYCOBACTERIUM CAPRAE GENOMES SHOW HIGH VARIABILITY IN THE REGION OF DIFFERENCE FOUR (RD4) AND ADJACENT SEQUENCES

S. Bröckl[1], R.K. Straubinger[2], M. Fink[3], S. Gellert[1], N. Ackermann[1], H. Blum[4], A. Graf[4], S. Krebs[4], M. Büttner[1]

[1]Bavarian Health and Food Safety Authority, LGL, Oberschleißheim, Germany, [2]Institute for Infectious Diseases and Zoonoses, Vet. Faculty, LMU Munich, Germany, [3]Ages, Austrian Agency for Health and Food Safety, Mödling, Austria, [4]Laboratory for Functional Genome Analysis (LAFUGA), Gene Centre, LMU Munich, Germany

Mycobacterium (M.) caprae can infect a wide range of domestic animals, wild-life species, and man and causes tuberculosis (TB) in cattle and red deer in the Alpine regions of Austria and Bavaria.
Molecular analysis including next generation sequencing (NGS) revealed that M. caprae genomes show substantial variation in their genomic region of difference four (RD4). This finding helped to differentiate *M. caprae* isolates and group them into three subtypes termed "Allgäu", "Karwendel" and "Lechtal", according to their geographical origin. The genomic variability of the RD4-region was used for PCR-based differentiation of recent *M. caprae* isolates from cattle (n=54) and red deer (n=61). Interestingly, a disproportionate occurrence of the "Lechtal" subtype was found in cattle (54%) and red deer (62%) in the Bavarian Alps. The genome of the "Lechtal" subtype lacks completely the RD4-region including flanking sequences (38 kb). The subtype "Karwendel" shows only a partial deletion of the RD4 region and so far has been exclusively detected in the Karwendel Mountains. The subtype "Allgäu" contains an intact RD4 region, but is represented less frequently in red deer (17%) and cattle (41%) in the Allgäu region. Two human patients suffering from a *M. caprae* infection were found to carry the "Allgäu" subtype with the conserved RD4-region using the PCR differentiation of the bacterial genomes. PCR fragments (1031 bp) from their central RD4 region revealed a 100% sequence identity with those of four randomly selected bovine "Allgäu" RD4 genomes. Finally, genomes of four additional *M. caprae* isolates could not be differentiated with the primers used for RD4 subtyping. Thus an even higher variability in and around this genomic region is likely. So far, NGS confirmed an extended deletion affecting RD4 and adjacent sequences even exceeding the analyzed "Lechtal" gap in the *M. caprae* genome. Genome wide plasticity of *M. caprae* strains and isolates will be subject of further research.

Board No: 48 *(New and Re-) Emerging Zoonotic Diseases*

THE NUCLEAR EXPORT PROTEIN OF H5N1 INFLUENZA A VIRUSES RECRUITS M1 TO THE VIRAL RIBONUCLEOPROTEIN TO MEDIATE NUCLEAR EXPORT

L. Brunotte[1], J. Flies[1], H. Bolte[1], P. Reuther[1], F. Vreede[2], M. Schwemmle[1]

[1]Department for Microbiology and Hygiene, Institute of Virology, Freiburg, Germany, [2]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Influenza A Viruses are negative strand RNA viruses causing severe respiratory disease in humans. Unlike most other RNA viruses, Influenza A Viruses replicate their genome in the nucleus of an infected cell, which requires nuclear entry and exit of incoming and newly synthesized viral ribonucleoproteins (vRNPs), respectively. To date, nuclear export of RNPs is described to be mediated by the viral matrix protein 1 (M1) and the nuclear export protein (NEP), which assemble a nuclear export complex in a daisy chain manner. In this complex M1 recognizes the newly synthesized RNP by binding to the nucleoprotein and viral RNA. The C-terminal domain of NEP then binds to the RNP-bound M1, while the N-terminal domain of NEP, which harbours two nuclear export signals (NESs), mediates the interaction with the cellular nuclear export factor CRM1, thereby gaining access to the cellular nuclear export pathway. However, despite its nuclear export function NEP was recently discovered as a cofactor of the viral polymerase able to regulate its activity by binding to the viral polymerase subunits. The observation that both interactions are mediated by the C-terminal moiety of NEP raised the question whether these two features of NEP are functionally linked. Here we show that the interaction between M1 and the vRNP depends on the presence of the C-terminal domain of NEP. Intriguingly, deletion of the last three amino acids of NEP not only abolishes the polymerase enhancing function of NEP but also abrogates its ability to mediate nuclear export of RNPs, indicating that both functions of NEP are coupled and that the association of NEP with the viral polymerase might also be essential for its nuclear export function. In conclusion, our data suggest a new refined model for the RNP nuclear export complex in which the C-terminus of NEP simultaneously interacts with the viral polymerase and M1, while the NES containing N-terminus is available for CRM1 binding.

Board No: 49 *(New and Re-) Emerging Zoonotic Diseases*

BAYESIAN MODELLING OF FACTORS POTENTIALLY INFLUENCING THE SPATIAL DISTRIBUTION OF *ECHINOCOCCUS MULTILOCULARIS* IN FOXES

F.J. Conraths[1], S. Schwarz[1], A. Sutor[1], L. Hoffmann[2], V. Schmid[3], C. Staubach[1]

[1]Institut Für Epidemiologie, Friedrich Loeffler Institut, Greifswald Isle of Riems, Germany, [2]Abteilung Veterinäruntersuchung, Landesamt Für Verbraucherschutz, Bad Langensalza, Germany, [3]Institut Für Statistik, Ludwig Maximilians Universität, München, Germany

Alveolar echinococcosis is considered one of the most dangerous autochthonous parasitic zoonoses in central Europe. The red fox (*Vulpes vulpes*) represents the main definitive host of *Echinococcus multilocularis* in Europe.

To investigate the potential influence of environmental factors on the spatial epidemiology of *E. multilocularis*, 38,446 foxes were sampled in two German Federal States (Brandenburg and Thuringia) and the results of the parasitological examination linked to a geographic information system. The landscape composition per spatial unit was derived from a high-resolution land-survey vector database and supplemented by a digital elevation model. Data were analyzed using a hierarchical Bayesian model.

On the municipality level and with environmental data at higher resolution, the study confirmed results of previous work, in which we had utilized exact locations of foxes and micro-habitat data. Furthermore, the preference of infected foxes for open landscapes with pasture was demonstrated in both regions despite the different land-use characteristics. However, prediction of endemic areas was not possible alone on the basis of land-use classes in the study areas, while neighbourhood to an area with *E. multilocularis* infections in foxes was a good predictor for the occurrence of the parasite.

We conclude that *E. multilocularis* is likely to spread to neighbouring areas more or less regardless of environmental factors that can influence the spatial distribution of the parasite, if the parasite is present in a region in Germany.

Board No: 50 *(New and Re-) Emerging Zoonotic Diseases*

COULD DOGS BE USEFUL FOR THE EPIDEMIOLOGICAL SURVEILLANCE OF WEST NILE VIRUS ACTIVITY?

B. Davoust[1], I. Leparc-Goffart[2], J. Demoncheaux[3], G. Trombini[3], O. Mediannikov[4], J. Marié[3]

[1]Unité De Recherche en Maladies Infectieuses Et Tropicales Emergentes (URMITE) CNRS UMR 7278 Ird 198 Inserm U1095 Aix Marseille Université, Faculté De Médecine, Marseille, France, [2]Centre National De Référence Des Arboviroses Institut De Recherche Biomédicale Des Armées, Service De Santé Des Armées, Marseille, France, [3]Groupe De Travail en épidémiologie Animale Du Service De Santé Des Armées, Service De Santé Des Armées, Toulon, France, [4]Unité De Recherche Sur Les Maladies Infectieuses Et Tropicales émergentes (IRD 198), IRD, Dakar, Senegal

To evaluate the presence and extension of West Nile virus (WNV) in South-East of France and in African areas, specific antibody prevalence was determined using ELISA and Western blot assays among 753 dogs. Results revealed a seroprevalence rate of 30.9% in Chad, 12.7% in Djibouti, 12.5% in Congo DR, 11.1% in Senegal and 0% in Gabon. The results and the statistical analysis reveal important differences in the seroprevalence rates, according to the geographic area. This situation could result from an ecosystem made of wet forests (Gabon) without migratory birds, unfavorable to the circulation of WNV. In France, dogs are the sentinels of virus circulation in the Var (12%) and Gard (9%). Moreover 12.5% of dogs imported from Hungary are seropositive. We also observed a seroconversion in dogs sent for a short mission in endemic countries for WNV, like Chad. Dogs could be used in the surveillance system of WNV infection in addition to the usual active surveillance concerning chickens or horses located in selected ecological sites or passive surveillance concerning dead birds. Even if still a matter of debate, dogs living close to humans could attract infected mosquitoes and prevent human infection (zooprophylaxis).

Board No: 51 *(New and Re-) Emerging Zoonotic Diseases*

A TRANSBOUNDARY SURVEILLANCE NETWORK TO MONITOR MOSQUITO-BORNE DISEASES IN THE MEDITERRANEAN AREA

R. Desiato[1], A. Pautasso[2], D.R. Francese[2], M. Ballardini[3], A. Accorsi[3], C. Colombino[4], M. Irranca[4], M. Obinu[4], P. Murtas[4], M. Selmi[5], J. Alfonsi[6], J.B. Santoni[6], C. Casalone[2], W. Mignone[3]

[1]Biostatistica, Epidemiologia Ed Analisi Del Rischio, Istituto Zooprofilattico Sperimentale Del Piemonte, Liguria E Valle D'aosta, Torino, Italy, [2]Neuroscienze Lab. Neuropatologia, Istituto Zooprofilattico Del Piemonte, Liguria E Valle D'aosta, Torino, Italy, [3]Sezione Di Imperia, Istituto Zooprofilattico Del Piemonte, Liguria E Valle D'aosta, Torino, Italy , [4]Settore Ambiente E Suolo, Provincia Di Oristano, Oristano, Italy , [5]Dipartimento Di Prevenzione, Asl 2 Lucca, Lucca, Italy, [6]Service Lav Démoustication, Conseil Generale De La Corse Du Sud, Ajaccio, France

In recent years Europe has experienced the introduction of 4 main mosquito-borne diseases (MBDs) of tropical origin: the zoonoses caused by West Nile (WNV), Usutu (USUV) Chikungunya (CHIKV) and dengue virus (DENV).

The establishment of the invasive mosquito *Aedes albopictus* in all Italian Regions (with the exception of Valle d'Aosta) from 1990 onwards, as well as in Southern France, has caused great concern because of its high vector competence for CHIKV and DENV. On the other hand, *Culex pipiens*, which is the main vector of WNV and USUV, is widely represented in all European countries.

Sardinia, Tuscany and Liguria in Italy, and Corsica in France, are important commercial and tourist regions located in the Mediterranean basin and some of them have recently experienced MBDs outbreaks.

The aim of this work is to present a transboundary surveillance network established in this Mediterranean area, and supported by the "Programme de Coopération Transfrontalière Italie–France Maritime 2007-2013", through the REDLAV project in order to improve our knowledge on mosquito distribution and to set up a plan of intervention in case of emergence of MBDs.

Entomological surveillance was carried out from 2011 to 2013 through adult mosquito collection, using georeferenced traps (CO2-baited CDC and BG sentinel traps, light traps), and through indirect estimation of the population density of *Ae. albopictus* using ovitraps.

Traps were located in the entire area during the vector season activity.

In this survey, a total amount of 9,632 adult mosquitoes were collected (among these, 5,320 *Cx. pipiens* – 55% of the total, and 2,565 *Ae. albopictus*, -27% of the total).

The ovitrap monitoring allowed evaluating mosquito density in order to support the disinfestation plan. A public awareness campaign was also performed by means of didactic material explaining the project goals and activities and good practices to reduce Ae. albopictus population: leaflets, a videoclip broadcasted by local TV networks and a website (http://www.redlav.com/it/home/)
The availability of continuous data on mosquito populations provides useful information for prevention and early intervention in case of an epidemic emergency.

Board No: 52 *(New and Re-) Emerging Zoonotic Diseases*

THE BURDEN OF BRUCELLOSIS IN EASTERN INDIA

D. Dutta, D. Chatterjee, U. Chakraborty, S. Das

Brucella Research Unit, Peerless Hospital, Kolkata, India

Although it is well known that brucellosis is highly endemic in Arabian countries but in developing South East Asian countries like India the burden of brucellosis in general population is largely unknown. This study was aimed to find out the real scenario of this disease in Eastern India with the city of Kolkata being the core centre for this study. Blood samples were collected from 395 persons randomly and the samples were processed to study Brucella antibody levels by SAT, IgG and IgM ELISA tests and also by RBT test. There were 204 (51.6%) males and 191 (48.4%) females; most of them were in the age group of 20-60 years (76.2%). Although there was only one animal handler (0.25%), there was previous history of animal contact in 55 persons (13.9%) and 42 (10.6%) persons were raw milk consumers. Geographical locations of the subjects indicated a gradual decline in the number of patients from the city of Kolkata (50.9%) to adjoining districts (28.1%) and to surrounding states (21.0%). Brucella antibody was detected significantly in 42 males and 37 females (20%). Among 79 positive cases, 58 persons (73.4%) belonged to 20-60 years age group. Out of 190 cases without any history of animal contact or raw milk consumption, 17 (8.9%) showed positive results while out of 98 cases with history of animal contact or raw milk consumption, 19 (19.4%) showed positive results. This indicates that animal contact or raw milk consumption is an important predisposing factor of brucellosis in this part of India. Samples collected from 3 districts (South 24 Parganas, North 24 Parganas and Howrah) showed significantly higher positive results in comparison to other areas indicating a possible endemicity of brucellosis in these areas of Eastern India.

Board No: 53 *(New and Re-) Emerging Zoonotic Diseases*

THE VAMPIRE BAT VIROME: EVOLUTIONARY IMPLICATIONS IN AN IMMUNOLOGICAL CONTEXT

M. Escalera[1], E. Loza Rubio[2], B. Taboada[3], C. Arias Ortíz[3], G. Czirjak[1], A. Greenwood[1]

[1]Department of Wildlife Diseases, Leibniz Institut Für Zoo Und Wildtierforschung Im Forschungsverbund, Berlin, Germany, [2]Centro Nacional De Investigaciones en Microbiologia Animal (Cenid Microbiologia Animal), Inifap, Mexico Df, Mexico, [3]Instituto De Biotecnologıa, Universidad Nacional Autonoma De Mexico, Cuernavaca, Morelos, Mexico

Surveillance of wildlife is an important strategy for detecting viruses with zoonotic potential that represent a health risk both for humans and other animals. Several viruses have been detected in different bat species and many of these have medical importance, such as SARS. However, viral surveillance studies are restricted on describing absence or presence of viruses and have been carried out mainly in insectivorous or fruit-eating bats of Asia. Little is known about the viral epidemiology and diversity among neotropical bats, in particular on the common vampire bat (Desmodus rotundus). Vampire bat feeding behavior involves close body contact with its food source, representing a route for pathogen transmission via direct exchange of body fluids. Additionally, signs of co-adaptation between viruses and their hosts and can be detected as molecular footprints both in the host' immune molecules and in the viral genomes. A recent study has shown that there is an association between polymorphisms in the innate immune Toll-like receptors (TLRs) and specific pathogen infections common to rodents, showing that the genetic variation in the TLRs of mammalian species can be largely influenced by pathogen interactions.

The aims of this study are to describe the virome of a sample population of several free-ranging bats from Mexico, focusing mainly on the vampire bat. Further, we will characterize the bat TLRs involved in viral sensing (TLR 3, 7 and 9) and search for evolutionary footprints (e.g. sites under positive selection) both in the TLRs and in the viral genomes to reveal co-evolutionary patterns, integrating the data obtained in an evolutionary context. Until now, 43 bat individuals have been captured in different states of Mexico. Viral detection and TLR characterization will be done using both standard PCR and metagenomic approaches. Co-adaptation signatures will be detected by using molecular evolutionary methods.

So far, PCR results have shown that 3%-12% of the 43 individual tested are positive for AdV, CoV-2A, CoV-2C and Herpesvirus. Also, we have detected a novel retrovirus in captive vampire bats. The results obtained will be compared to the NGS data during the second phase of the project.

Board No: 54 *(New and Re-) Emerging Zoonotic Diseases*

SINGLE DOSE CONTRACEPTIVE RABIES VACCINE

M. Faber

Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, United States

World-wide elimination of dog rabies will only be possible through vaccination combined with control of the dog population. To achieve both objectives at the same time, we have developed a rabies and contraceptive immunization protocol based on the rabies variant TriGAS. This attenuated rabies virus (RABV) is non-neuroinvasive, and nonpathogenic even for immunocompromised mice. Intramuscular administration of a single dose of TriGAS triggers in mice a strong and sustained immune response not only against RABV but also to co-administered unrelated antigens such as ovalbumin. We found that this response is driven by transient TriGAS replication in the draining lymph nodes where the highest RABV mRNA levels were detected in B cells and dendritic cells (DCs). Based on these findings, we designed a contraceptive rabies vaccine consisting of a mix of TriGAS and gonadotropin-releasing hormon (GnRH) coupled to keyhole limpet hemocyanin (KLH). While administration of a single dose of GnRH-KLH in the absence of TriGAS was poorly or not immunogenic, the mixture of TriGAS and GnRH-KLH induced a strong, long-lasting antibody response to GnRH as well as to RABV. Mice immunized with TriGAS/ GnRH-KLH did not develop any clinical signs during an observation period of over three months suggesting that this vaccine is safe and has utility as an effective contraceptive rabies vaccine.

Board No: 55 *(New and Re-) Emerging Zoonotic Diseases*

HOST ASSOCIATION AND SPILLOVER OF BANK VOLE HEPACIVIRUS, GERMANY

S. Fischer[1], B. Hoffmann[2], N. Kratzmann[1], J.F. Drexler[3], M.H. Groschup[1], C. Drosten[3], M. Beer[2], R.G. Ulrich[1]

[1]Institute of Novel and Emerging Infectious Diseases, Friedrich Loeffler Institut, Federal Institute for Animal Health, Greifswald, Germany, [2]Institute for Diagnostic Virology, Friedrich Loeffler Institut, Federal Institute for Animal Health, Greifswald, Germany, [3]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Hepatitis C is a human disease, caused by the hepatitis C virus, frequently resulting in chronic infections leading to cirrhosis and hepatocellular carcinoma. A opportunistic virus hunting approach in 4770 rodents resulted in the identification and genome characterization of a novel hepacivirus, associated to the bank vole *Myodes glareolus* (Drexler et al., 2013).
Within the network "Rodent-borne pathogens" a monitoring of rodents and other small mammals is performed since spring 2010 at selected sites in Baden-Wuerttemberg, North Rhine Westphalia, Thuringia and Mecklenburg-Western Pomerania. As expected, bank voles and yellow-necked mouse were mainly trapped in forest habitats. In contrast, trapping in grassland habitats resulted mainly in trapping of common voles. In a pilot study, the novel hepacivirus was almost exclusively detected in bank voles suggesting a corresponding host specificity. In addition, hepacivirus RNA was detected in a few, most likely spillover-infected individuals of other rodent species.
In conclusion, the previously identified novel hepacivirus might have a host specificity for the bank vole with only few spillover infections. Future investigations will have to prove the molecular basis of the host specificity of this hepacivirus. The bank vole-associated hepacivirus may allow the development of a novel animal model for human hepatitis C.
Drexler et al., 2013. Evidence for novel hepaciviruses in rodents. PLoS Pathog. 9(6):e1003438

Board No: 56 *(New and Re-) Emerging Zoonotic Diseases*

ISOLATION AND CHARACTERIZATION OF A COWPOX VIRUS DERIVED FROM ITS SUPPOSED NATURAL RODENT RESERVOIR HOST

D. Hoffmann[1], J. Schluckebier[1], A. Franke[1], R. Ulrich[2], M. Jenckel[1], B. Hoffmann[1], M. Beer[1]

[1]Friedrich Loeffler Institut, Institute of Diagnostic Virology, Greifswald Isle of Riems, Germany, [2]Friedrich Loeffler Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald Isle of Riems, Germany

Poxviruses have plagued human mankind for more than ten thousand years and claimed the lives of millions. Although the WHO declared smallpox, the deadliest poxvirus, eradicated in 1980, infections with closely related orthopoxviruses are still reported. Notably, cowpox virus infections saw a steep rise in recent years. Cowpox virus (CPXV) enters the human population mostly via direct contact to companion animals, especially cats and pet rats. Serological findings, however, have pointed to wild rodents as the main virus reservoir. Yet the actual isolation of CPXV from this source has hardly ever been achieved. As further proof for the reservoir hypothesis we present here a CPXV strain isolated from the liver of a feral common vole (Microtus arvalis).

First, we used next generation sequencing to obtain the full-length DNA sequence of this CPXV strain and compared it with a reference CPXV isolated from a 2009 pet rat/human outbreak in Germany that showed high virulence in the affected animals. We then characterized the pathogenicity of the vole strain in its natural host as well as in Wistar rats. Both the common voles and the Wistar rats were experimentally inoculated with high and low titers of the CPXV vole isolate. Another group of voles was also infected with the 2009 CPXV rat strain. The vole strain caused no to only mild clinical symptoms in its natural host, while all Wistar rats developed respiratory symptoms followed by rashes. Common voles infected with a high titer of the rat strain virus showed severe signs of respiratory disease but no skin lesions, whereas infection with the low titer lead to excretion of virus but with reduced clinical signs.

This study reveals the susceptibility of the common vole to different CPXV strains - ranging from a well-adapted virus, which causes only slight clinical symptoms in the host organism, to a highly virulent strain. The low pathogenicity of the vole isolate in its eponymous host also provides evidence for the common vole actually being a reservoir for CPXV.

Combined with full genome data and the Wistar rat model, virulence studies in the common vole will facilitate future research on the correlation of genotype and pathotype of CPXV infections as well as the epidemiological role of the rodent reservoir and the zoonotic risk that emanates from these viruses.

Board No: 57 *(New and Re-) Emerging Zoonotic Diseases*

STUDIES ON ORTHOPOXVIRUS PATHOGENESIS AND VACCINE EFFICACY ASSESSMENT USING THE CALPOX-MARMOSET MODEL

L.L. Gan[1], T. Shi[1], M. Hoffmann[1], D. Motzkus[1], B. Neumann[1], C.P. Czerny[2], F.J. Kaup[3], K. Mätz-Rensing[3], Z. Chen[4], H. Ellerbrok[5], C. Stahl-Hennig[1]

[1]Unit of Infection Models, German Primate Center, Göttingen, Germany, [2]Department of Animal Sciences, University of Göttingen, Göttingen, Germany, [3]Pathology Unit, German Primate Center, Göttingen, Germany, [4]Aids Institute and Department of Microbiology, University of Hong Kong, Hong Kong, China, [5]Robert Koch Institute, Berlin, Germany

Keywords: orthopoxvirus, vaccination, animal model

Orthopoxviruses, e.g. smallpox (SPXV) or monkeypox virus (MPXV), are zoonotic pathogens that have led (SPXV) or still lead (MPXV) to human infections. In the case of SPXV, vaccination with vaccinia virus (VV) led to the eradication of the causative agent. Nevertheless, orthopoxviruses and their impact on public health are still of major interest, since there is common concern that they could be used as a bioweapon. Therefore, there is a general need for the development and evaluation of safe vaccines.
During a recent fatal outbreak in a group of common marmosets (*Callithrix jacchus*) kept in captivity, a so far unknown orthopoxvirus belonging to the cowpox group and designated calpox virus (CalPXV), has been identified. The marmoset is a well-studied animal model and infection with CalPXV represents a suitable platform for the validation of vaccines against orthopoxviruses.
In a previous study, we investigated the vaccine efficacy of VV Lister-Elstree (LE), modified VV Ankara (MVA), and modified VV Tiantan (MVTT) given intradermally, intramuscularly and intranasally, respectively, against CalPXV challenge. When the challenge was performed four weeks after the last immunization, we observed no protection after MVA vaccination, 25% protection following VV-LE vaccination, and the highest one following vaccination with MVTT (75 %). In addition, we found evidence that CalPXV replication starts locally in the nasal-associated-lymphoid-tissue (NALT) and tonsils, before it spreads to draining lymph nodes and then systemically as monitored by qPCR.
In an ongoing follow-up study, we want to expand our investigations on (i) the refinement of possible vaccination strategies to achieve 100% protection and (ii) the identification of immune correlates of protection.

For this purpose, we not only modified our study design by prolonging the time between vaccination and CalPXV challenge from four to ten weeks, but also included another mucosal vaccination route for MVTT, i.e. perorally through spray application. This study is in progress and will provide valuable information about the immune responses to MVTT vaccination and the potential of MVTT to be used as a safe vaccine against orthopoxvirus infection.

Board No: 58 *(New and Re-) Emerging Zoonotic Diseases*

WHOLE GENOME COMPARISON OF *ESCHERICHIA COLI* SEQUENCE TYPE 131 (ST131) FROM HUMAN AND ANIMAL ORIGIN TO DETECT A POSSIBLE ZOONOTIC TRANSMISSION OF AN EMERGING MULTIDRUG-RESISTANT PATHOGEN IN GERMANY

H. Ghosh[1,2,3], L. Falgenhauer[2,3], M. Fritzenwanker[2,3], Y. Yao[2,3], C. Imirzalioglu[2,3], T. Chakraborty[2,3]

[1]Justus Liebig Universität Gießen, Institute of Medical Microbiology, Gießen, Germany, [2]Institute of Medical Microbiology, Justus Liebig University Giessen, Giessen, Germany, [3]German Center for Infection Research (Dzif), Partner Site Giessen Marburg Langen, Justus Liebig University Giessen, Giessen, Germany

Background: *Escherichia coli* sequence type 131 (*E.Coli* ST131) is a recently globally disseminating pandemic super clonal group, causing a serious problem in human and veterinary medicine with undefined reservoirs and transmission pathways. *E.coli* ST131 is notable because it is often associated with fluoroquinolone resistance and extended-spectrum beta-lactamase (ESBL). ESBL genes confer resistance to third-generation-cephalosporins such as cefotaxime which leads to less treatment options and causes higher morbidity, mortality and treatment costs in clinical settings. The aim of this study is to find out the clinical epidemiology of *E. coli* ST131 in terms of antimicrobial resistance and virulence gene in different host.
Materials and Methods: A subset of 15 ST131 isolates (n=14 human, n=1 dog) was selected from a large set of ESBL producing *E. coli* from humans and companion animals. Preliminary molecular characterization was performed including the determination of the CTX-M alleles and phylogenetic groups using PCR. Whole-genome sequencing of all the strains was performed to identify relationships between these isolates. Comparative analysis of these isolates together with recently published ST131-*E.coli* genome was performed in order to understand the phylogenetic relationship between isolates infecting humans and companion animals. FimH-based subtyping and determination of the resistance gene content was performed using database search.
Results: Analysis of the phylogenetic groups showed that group B2 was the prevalent phylogenetic group (67%, n=10), followed by phylogenetic groups D (27%, n=4) and A (6%, n=1). Investigation of fimH (gene encoding type 1 fimbrial adhesin) revealed two variants of fimH in the subset (H30 and H41), indicating that the investigated strains might have originated from two widely dispersed clones.

Dog isolate was closely related to human inpatient isolates. Although most of the strains from animal and humans maintained a distinct lineage, few of them are interspersed.

Conclusion: In our study, the phylogeny of ESBL-encoding *E.coli* ST131 was examined on a genomic level. The results suggest that companion animals might act as a reservoir for human infection or vice versa.

Board No: 59 *(New and Re-) Emerging Zoonotic Diseases*

RACCOON DOG (*NYCTEREUTES PROCYONOIDES*) AS A RESERVOIR OF FOUR TRICHINELLA SPECIES

P. Heikkinen, H. Hietala, M. Isomursu, V. Hirvelä-Koski

Research Department, Finnish Food Safety Authority, Oulu, Finland

Trichinella infections are very common in Finnish wildlife. Four species have been detected including *T. nativa, T. spiralis, T. britovi* and *T. pseudospiralis*. The raccoon dog plays an important role in the epidemiology of trichinellosis. In the Finnish monitoring program, 30 % of raccoon dogs were positive for trichinellosis in 2006-2012 (N=1533). In this study we examined the distribution of different *Trichinella* species in the infected raccoon dogs.

Trichinella genotypes were identified in samples of 194 raccoon dogs using multiplex PCR method. A pool of five larvae was examined from each animal. Acceptable result was obtained from a total of 172 animals. PCR products could not be observed in 22 samples.

The most common species was *Trichinella nativa*. It was detected in 96.5 % of the samples. *T. pseudospiralis, T. spiralis* and *T. britovi* were found in 4.1 %, 3.5 % and 1.7 % of the samples, respectively. Mixed infections of two *Trichinella* species were detected in 5.8 % of the positive animals. The freeze-tolerant *T. nativa* is a species adapted to arctic climate whilst *T. britovi* is the most common species in wildlife in temperate climate south of Finland. The only non-capsulated species, *T. pseudospiralis* can infect birds. Wild and domestic pigs are the main hosts of *T. spiralis*. All four species are infective to humans.

T. nativa was observed in raccoon dogs in all parts of Finland, whereas *T. spiralis* and *T. britovi* were present only in eastern and south-eastern parts of the country. *T. britovi* was not found north of the 63rd parallel. *T. pseudospiralis* infections were found from eastern and south-eastern areas, but also from southern parts of Finland.

Although trichinellosis has been in recent years very rare in domestic pigs in Finland, it should be kept in mind that Finnish wildlife is a permanent reservoir of four *Trichinella* species including *T. spiralis*, the most common species in pigs.

Board No: 60 *(New and Re-) Emerging Zoonotic Diseases*

EFFECTS OF THE ZOONOTIC ROUNDWORM LARVAE *TOXOCARA CANIS* AND *TOXOCARA CATI* ON MYELIN MARKER GENE TRANSCRIPTIONS AND CNS CELL SURVIVAL

L. Heuer[1], M. Beyerbach[2], A. Beineke[3], C. Strube[1]

[1]Institute of Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany, [2]Department of Biometry, Epidemiology and Information Processing, Who Centre for Research and Training in Veterinary Public Health, University of Veterinary Medicine Hannover, Hannover, Germany, [3]Department of Pathology, University of Veterinary Medicine Hannover, Hannover, Germany

Neurotoxocarosis is an infection of the central nervous system by migrating larvae of the zoonotic roundworms *Toxocara canis* and *T. cati*. This infection results in histopathological changes, e.g. demyelination. The underlying molecular mechanisms of these myelin losses are unknown. Therefore, the transcription rates of different myelin marker genes were investigated in cerebra and cerebella of C57BL/6J mice at time points 28, 42, 70, 98, 120 and 150 days post infection (dpi) using probe-based quantitative real-time PCR. Investigated genes encode classical myelin proteins such as MAG (myelin-associated glycoprotein), MBP (myelin basic protein), MOG (myelin/oligodendrocyte glycoprotein), PLP1/DM20 (proteolipid protein) and CNP (2',3'-cyclic-nucleotide-3'-phosphohydrolase) as well as the associated regulatory factors Mrf (myelin gene regulatory factor), Olig2 (oligodendrocyte transcription factor 2) and NogoA (neurite outgrowth inhibitor). After normalization against evaluated reference genes and correction for PCR efficiency, significant differences were detected for transcription rates of all investigated genes. Most transcription changes in the cerebellum were detected for *mbp* and in the cerebrum for *cnp*. Most gene specific transcription rates changed at time point 120 dpi in the cerebrum and at time point 70 dpi in the cerebellum. At 28 dpi, effects could be observed for *cnp*, *mog* and *mag*. Compared to the uninfected control group, *cnp* and *mog* were upregulated in the cerebra of both *T. canis* and *T. cati* infected mice. Transcription of *mag* was upregulated exclusively in the cerebella of *T. canis* infected mice 28 dpi. Later, 42 and 98 dpi *mag* is upregulated in both cerebra and cerebella of *T. canis* infected mice. As*mag* indirectly activates myelogenesis, upregulation might indicate reactive gene transcription after myelin damage by *T. canis* larvae. This is supported by the histological finding that parenchymal destruction was more pronounced in *T. canis* than in *T. cati* infected mice.

For further characterization of possible pathomechanism, murine neuronal cells (CAD cells) were co-cultivated with 1000 larvae of *T. canis* or *T. cati*, respectively. After seven days cell viability was evaluated using a dead/live stain. Presence of larvae did not have any effect on the vitality of undifferentiated CAD cells. Further investigations regarding the effect of larvae on other cell lines and brain tissue cultures (brainslices) are currently in progress.

Board No: 61 *(New and Re-) Emerging Zoonotic Diseases*

CHARACTERIZATION OF HOST–PATHOGEN INTERACTIONS OF HUMAN MONOCYTE-DERIVED MACROPHAGES INFECTED WITH *C. MURIDARUM* OR *C. PSITTACI*

K. Hillarius[1], D. Barz[2], K. Sachse[1], A. Berndt[1]

[1]Imp, Friedrich Loeffler Institut, Federal Institute for Animal Health, Jena, Germany, [2]Institute for Transfusion Medicine, University Hospital of Jena, Jena, Germany

Chlamydia species are ubiquitously spread over the world and can infect animals and humans. The disease pattern depends on the chlamydial species that infects a distinct host. Many chlamydiae are host adapted (e.g. *C. muridarum* in rodents), but some, such as *C. psittaci*, have a broader host range and cause diseases in different animal species and humans.

In the present study, we characterized the interaction of *C. muridarum* and *C. psi*ttaci with human monocyte-derived macrophages by examination of the immune response of macrophages and chlamydial propagation.

Infection of macrophages with both chlamydial species [MOI (multiplicity of infection)1, 2 and 5] resulted in significantly higher numbers of inclusion-forming units per cell (ifu/cell) of *C. psittaci* at 24 and 48 hours post infection (hpi). Remarkably, at 4 hpi, *C. muridarum* infection led to significant higher ifu/cell.

Flow cytometric analysis revealed the tendency of macrophages to develop a M1-phenotype at 48–72 hpi of *C. psittaci* infection. In contrast, *C. muridarum* infection led to a significant increase of CD206, typically found on M2 macrophages, at 48–72 hpi. Significant differences in the stimulation capacity were also seen for CD14, MHCI, MHCII, CD40 and TLR-4.

Release of soluble effectors of the respiratory burst reaction, such as reactive oxygen species (ROS), superoxide (SOX) and nitric oxide (NO) was low, but showed interesting tendencies. After 4 hpi, *C. psittaci* released significantly more ROS than *C. muridarum*. In *C. muridarum* infection, the highest ROS release occurred at 24 hpi. Both chlamydial species released most SOX after 4hpi. NO release was significantly higher in *C. muridarum* high (samples divided in high and low responders) infection than in *C. psittaci* infection after 24–48 hpi.

The data show that both chlamydial species were able to propagate in human monocyte-derived macrophages, but had different effects on the host's immune response.

Board No: 62 *(New and Re-) Emerging Zoonotic Diseases*

ZOONOTIC PATHOGENS ISOLATED AND DETECTED FROM REGIONAL ANIMAL SHELTER AND ITS VICINITY

P.C. Hsueh, M.J. Pan

Graduate Institute of Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan

Zoonoses are infections naturally transmissible between vertebrate animal hosts and humans. Many zoonotic infections may be contracted from the workplace; potential agents include bacteria, viruses, fungi and helminthes. The aim of this study was to estimate the prevalence of selected zoonotic pathogens from regional animal shelter with reference to their zoonotic risk. In order to isolate leptospires, we collected 48 samples from environmental soil, ditch water and wild rodent kidney. All samples were inoculated into Korthof's medium with anti-microbial supplements. Positive isolates were confirmed with microscopic agglutination test (MAT) against a panel of reference antisera and by polymerase chain reaction (PCR). Fifty stool specimens from stray dog in the shelter was collected for detecting internal parasites by examination the presence of eggs using flotation technique. Base on dark field microscope examination, *Leptospira* spp. was found in 20% (2/5) water, 0% (0/4) soil and 12.8% (5/39) rodent kidney samples. Out of seven positive cultures, two from rodent kidney were confirmed as Leptospira Javanica serogroup by both MAT and PCR. Fecal examination revealed *Toxocara canis* eggs in 50% (25/50) and Ancylostoma caninum 10% (5/50). In conclusion, we demonstrate that zoonotic leptospirosis and roundworms were highly prevalent among wild rodents and stray dogs in animal shelter. Infection control and prevention program should be developed to minimize the introduction and spread of zoonotic pathogens. These measures protect not only the animals within the shelter, but also the people working with and/or adopting the animals.

Board No: 63 *(New and Re-) Emerging Zoonotic Diseases*

FACTORS INFLUENCING THE CIRCULATION OF PARASITIC ZOONOSES IN SPECIFIC CONDITIONS OF THE TATRA NATIONAL PARK, SLOVAKIA

Z. Hurníková[1,2], M. Miterpáková[1], B. Chovancová[3]

[1]Dept. of Parasitic Diseases, Institute of Parasitology SAS, Košice, Slovakia, [2]Dept. of Parasitology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia, [3]Dept. Of Zoology, Research Station and Museum of Tanap, Tatranská Lomnica, Slovakia

The Tatra National Park (TANAP) represents in the long term the most important recreational area in Slovakia. From a public health perspective, the monitoring of serious parasitic diseases transmissible to humans in their natural reservoirs is of great importance.

The aim of our work was to study host-parasite-landscape interactions in specific environmental conditions in areas of TANAP. More than 200 wild carnivores originated from various recreational localities were examined for the presence of *Echinococcus multilocularis*, *Trichinella* spp., *Dirofilaria* spp. and intestinal helminthes of zoonotic importance. All samples came from the death animals and were collected by TANAP workers. Small intestines of carnivores were examined for the presence of *E. multilocularis* using modified sedimentation and counting technique; muscle samples were examined individually for the presence of *Trichinella* spp. larvae by the artificial HCl-pepsin digestion and species identification was performed by means of PCR developed by Pozio and La Rosa (2003). DNA isolated from spleens was remit to PCR analysis for diagnosis of dirofilariosis according to Rishniw et al. (2006). Fecal samples were investigated using coproscopic flotation methods.

The presented research work revealed the presence of *E. multilocularis* tapeworm in almost 40 % of foxes from TANAP and adjacent areas. *T. britovi* larvae harbored over 19 % of foxes and also 19.4 % of predators from the family Mustelidae (19.4 %). Dirofilariosis has also been found to be circulating in the studied area. *D. repens* was identified in 24.6 % of investigated foxes and in a beech marten. Moreover, examination of the fecal samples of free living carnivores revealed 58.3 % of them being infected with at least one zoonotic parasite (Taeniidae, *Dipylidium caninum*,*Trichuris vulpis*, *Toxocara canis*, *Toxascaris leonina*, *Strongyloides stercoralis*, *Ancylostoma* spp.).

Zoonotic nature of the parasitic diseases in TANAP as the most visited tourist area in Slovakia greatly increases the risk of transmission to humans. Our results point out the need to comply with hygiene rules as the most effective measure for prevention of infection.

The work was supported by VEGA 1/0702/12 and APVV 0605-12.

Board No: 64 *(New and Re-) Emerging Zoonotic Diseases*

DO INSECTIVORE BATS POSE A POTENTIAL FOR ZOONOTIC PARASITE TRANSMISSION?

Z. Hurníková[1,2], A. Kočišová[2], A. Ondrejková[3], D. Löbbová[4], E. Hapl[4]

[1]Dept. of Parasitic Diseases, Institute of Parasitology SAS, Košice, Slovakia, [2]Dept. of Parasitology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia, [3]Dept. Of Epizootology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia, [4]Research and Conservation, Slovak Bat Conservation Society, Bardejov, Slovakia

Bats serve as the final or intermediate host to a great diversity of ecto- and endoparasites. Because this animal group is isolated from other vertebrate species ecologically and behaviourally, their parasite species and communities are specific and quite distinct from those of other vertebrates. Epizootological situation on parasites incidence in populations of insectivore bats from European territory is not investigated and existing parasitological research in these host groups is only sporadic and mainly of local significance.

The objective of the pilot study was to ascertain the composition of parasite fauna in individual bat species populations for further study of the host – parasite relationship with regard to ecological and anthropic interactions with bat populations.

Ecologic and epidemiological monitoring and selection of research localities has been provided in winter 2013/2014 under license of Slovak Ministry of Environment in cooperation with members of Slovak Bat Conservation Society and with Slovak State Nature Protection. For parasitological investigation, the bat droppings were collected from wintering areas in the bat caches and sampled during trappings. Ectoparasites were collected by combing or "clean bag method". The bat faeces were investigated for the presence of parasite eggs and protozoan oocysts using standard flotation methods.

In total 88 faecal samples from bats belonging to 12 species were examined coprologically. The presence of parasites was ascertained in 64.60 % of specimens. Most frequent were Eimeria oocycsts (61.36 %). In 9.09 % of samples eggs of nematodes belonging to suborder Spirurina and in 2.27 % eggs from subfamily Capillariinae were detected. The hymenolepidid cestode eggs were present in two individuals (1.14 %) from one locality.

In total 328 ectoparasites that belonged to 5 families and 5 genera of ticks or insects were recorded. Ixodes verspertilionis (1.72 %), Steatonyssus occidentalis (5. 17 %), Spinturnix mystacina (79. 31 %), S. bechsteini (20. 68 %) ticks were detected and from the Diptera Stylidia biarticulata (Nycteribiidae) (12.1%) and one species of fleas, Ischnopsyllus intermedius (3.4%).

The pilot study does not uniquely revealed the presence of zoonotic agents in the sampled group of bats. However, more substantial knowledge on the parasite fauna composition requires long term and systematic research, focused on detailed identification of parasites obtained from dead individuals, including also the diagnosis of blood parasites is needed to evaluate the transmission risk of zoonotic parasites.

The work is funded by APVV-0605-12 project.

Board No: 65 *(New and Re-) Emerging Zoonotic Diseases*

HIGH SEROPREVALENCE OF *BORRELIA MIYAMOTOI* ANTIBODIES IN FORESTRY WORKERS AND INDIVIDUALS SUSPECTED OF HUMAN GRANULOCYTIC ANAPLASMOSIS IN THE NETHERLANDS

S. Jahfari[1], T. Herremans[2], A.E. Platonov[3], H. Kuiper[4], L.S. Karan[3], O. Vasilieva[3], J.W.R. Hovius[4], M.P.G. Koopmans[5], H. Sprong[2]

[1]Laboratory for Zoonoses and Enviromental Microbiology, Rivm / Erasmus Rotterdam, Bilthoven, Netherlands, [2]Centre for Infectious Disease Control Netherlands, National Institute for Public Health and Environment (Rivm), Bilthoven, Netherlands, [3]Research and Epidemiology Unit, Research Institute of Epidemiology, Moscow, Russian Federation, [4]Department of Internal Medicine, Section of Infectious Diseases, Academic Medical Centre, Amsterdam, Netherlands, [5]Department of Virology, Erasmus Mc, Rotterdam, Netherlands

Substantial exposure to *Borrelia miyamotoi* occurs through bites from *Ixodes ricinus* ticks in the Netherlands, which also transmit *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum*. A flu-like illness with high fever, resembling Human Granulocytic Anaplasmosis, has been attributed to Borrelia miyamotoi-infections. *Borrelia miyamotoi*-infections associated with chronic meningoencephalitis have also been described in case reports.

Aims: The long-term objective of our studies is to gain more insight in the public health risk of *B. miyamotoi*. As a first attempt to describe the exposure of *B. miyamotoi* in the Netherlands, using a newly developed serological assay, we determined here the seroprevalence of anti-*B. miyamotoi* antibodies in different risk groups within the general population.

Methods and results: Assuming that an IgG antibody response against *Borrelia miyamotoi* antigens reflects (endured) infection, the seroprevalence in different risk groups was examined. Antibodies to the GlpQ protein of *B. miyamotoi* were determined by an in house Luminex-assay. Sera from nine out of ten confirmed *Borrelia miyamotoi* infections from Russia were found positive, and no significant cross-reactivity was observed in secondary syphilis patients. The seroprevalence in blood donors was set at 2.0% (95% CI 0.4–5.7%). Elevated seroprevalences in erythema migrans patients 5.6% (3.0-9.2%) and in individuals with serologically confirmed 7.4% (2.0-17.9%) or unconfirmed 8.6% (1.8-23%) Lyme neuroborreliosis were not significantly different from blood donors. The prevalence of anti-*Borrelia miyamotoi* antibodies among forestry workers 10% (5.3-16.8%) and in patients with serologically unconfirmed but suspected Human Granulocytic Anaplasmosis 14.6% (9.0-21.8%) were significantly higher compared the seroprevalence in blood donors.

Conclusions: Our findings indicate that infections with *Borrelia miyamotoi* occur in tick-exposed individuals in the Netherlands. In addition, *Borrelia miyamotoi* infections should be considered in patients reporting tick bites and febrile illness with unresolved aetiology in the Netherlands, and other countries where *Ixodes ricinus* ticks are endemic.

Board No: 66 *(New and Re-) Emerging Zoonotic Diseases*

NEXT GENERATION SEQUENCING (NGS)-BASED CHARACTERIZATION OF THE MUSTELID HERPESVIRUS-1 (MUSHV-1) GAMMAHERPESVIRUS FROM EUROPEAN BADGER (*MELES MELES*)

A.A. Murphy[1], L. Davison[1], M. Emery[1], R. Belshaw[1], S. Grierson[2], D. Delahay[2], S. Lesellier[2], M. Chambers[2], B. Ehlers[3], N. Lyons[1], M.A .Jarvis[1]

[1]School of Biomedical and Health Care Sciences, University of Plymouth, Plymouth, United Kingdom, [2]Animal Health and Veterinary Laboratories Agency, Weybridge, Surrey, United Kingdom, [3]Division 12 'Measles, Mumps, Rubella and Viruses Affecting Immunocompromised Patients, Robert Koch Institut, Berlin, Germany

Mustelid herpesvirus 1 (MusHV-1) is a gammaherpesvirus of the *Percavirus* genus that was originally isolated from lung tissue of a young adult female European badger (*Meles meles*) in the Southwest of England. MusHV-1 has subsequently been shown to be ubiquitous in all badger populations studied, and results in an apparently benign infection. To date, genomic sequence is known for only a small region corresponding to the DNA polymerase and glycoprotein B genes. Based on phylogenetic analysis MusHV-1 has been identified as the prototypic member of a closely related group of carnivore percaviruses found in additional mustelids (otters and fisher), canids (Darwin's fox) and felids (domestic cats, bobcats and puma); these carnivore gammaherpesviruses are more distantly related to the *Percavirus* type species, equine herpesvirus 2 (EHV-2). Using next generation sequencing (NGS) technology combined with PCR and Sanger–based methodology we have resolved the MusHV-1 genome to a single contig of 112,239 bp. Sequence reads from two independent experiments were assembled using the de-novo assembly tool of CLC Genomics Workbench. BLASTX was used to identify contigs containing related herpesvirus genome, reducing the number of contigs of interest from twenty-one to three. These three contigs were then assembled by alignment to the EHV-2 genome – the closest known fully sequenced virus. An internal repeat region, between open reading frames (ORFs) 70 and 74, was resolved by using PCR and Sanger–based sequencing. The 112,239 nucleotide long contig contains 156 ORFs. The overall genome organisation is similar to EHV-2, with several blocks of conserved genes, but there are some differences. A number of ORFs in the left arm are absent from the MusHV-1 sequence. MusHV-1 unique ORFs potentially involved in immune and host response modulation were also identified.

Badgers are a major reservoir species for transmission of mycobacterium bovis (Mb) to cattle, and we are currently developing MusHV-1-based vectors as 'disseminating' vaccines against Mb infection in badger populations. In addition to basic questions of gammaherpesvirus biology and host interaction, annotation of the MusHV-1 genome will enable prototype MusHV-1-based Mb vaccine vector construction for initial immunogenicity studies in badgers.

Board No: 67 *(New and Re-) Emerging Zoonotic Diseases*

DISSEMINATING HERPESVIRUS-BASED STRATEGIES TO TARGET EBOLA VIRUS IN INACCESSIBLE WILD GREAT APE POPULATIONS

A. Marzi[1], A.A. Murphy[2], C. Parkins[3], M. Emery[2], I. Messaoudi[4], H. Feldmann[1], M.A. Jarvis[2]

[1]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, United States, [2]School of Biomedical and Health Care Sciences, University of Plymouth, Plymouth, United Kingdom, [3]Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton, Oregon, United States, [4]Division of Biomedical Sciences, University of California, Riverside, United States

Ebolavirus (EBOV) causes rapidly progressing hemorrhagic fever in infected humans (mortality rates >90%). Great apes are similarly highly susceptible to EBOV, and are a major transmission source during human EBOV outbreaks in Africa. A number of effective EBOV vaccines have been developed. However, these vaccines require direct inoculation for induction of EBOV immunity. Therefore, their ability may be limited for providing high levels of coverage for inaccessible wild great ape populations. We hypothesize that a novel 'disseminating' vaccine based on cytomegalovirus (CMV) vectors expressing EBOV antigens may achieve the desired levels of coverage. In this scenario, the capacity of the 'disseminating' vaccine to spread through the targeted population by animal-to-animal contact following inoculation of a few 'founder' animals is used to confer EBOV-specific immunity at the population level. We have shown the capacity of a CMV-based EBOV vaccine to provide durable protective immunity to lethal EBOV (*Zaire*; ZEBOV) challenge in mice (Tsuda et al. 2011). We have subsequently constructed RhCMV/ZEBOV vectors using a RhCMV BAC containing the full length RhCMV genome. ZEBOV ORFs were inserted in place of a non-essential RhCMV ORF by lambda-based linear recombination. Viruses were reconstituted by electroporation into rhesus fibroblasts and western analysis of infected cell lysates using monoclonal ZEBOV and epitope tag-specific antibodies confirmed expression of ZEBOV proteins and stability of expression over multiple passages. DNA sequencing of BAC and reconstituted viral DNA confirmed integrity of the RhCMV vectors. We recently initiated studies in rhesus macaques to determine the capacity of CMV-based vaccines expressing the full-length ZEBOV glycoprotein to provide protective immunity in the ZEBOV macaque model, which is regarded as the 'gold standard' model for EBOV vaccination studies.

In addition to immunogenicity/efficacy, the level of RhCMV/ZEBOV shedding into saliva and urine is a critical parameter that will be monitored to assess the disseminating potential of the vaccine. Upon demonstration of efficacy by direct inoculation, future studies in the ZEBOV macaque model will assess the protective capacity of immunity conferred by animal to animal spread of the vaccine.

Andrea Marzi and Aisling A. Murphy contributed equally to this work.

Board No: 68 *(New and Re-) Emerging Zoonotic Diseases*

DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST398 IN MEAT AT RETAIL IN THE CZECH REPUBLIC

R. Karpišková, K. Koukalova, I. Kolačková

Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been described in food-producing animals including pigs, bovine and poultry. The predominant LA-MRSA in Europe belongs to the clonal complex (CC) 398. This type of MRSA in pig was first reported in France and later this MRSA clonal complex was also observed in several European countries, in North America and Asia. MRSA in livestock poses a risk of spreading not only to other animals at farm level and while slaughtering, but also during meat processing and at retail. The aim of this study was to monitor the occurrence of MRSA in poultry, beef and pork meat and liver at retail market in the Czech Republic. Altogether 334 samples (100 poultry, 79 beef, 155 pork) were collected and manufactured in accordance with Commission Decision 2008/55/EC. Suspect colonies were analyzed by the PCR method for detection of *S. aureus* specific fragment and screened for the presence of *mecA/ mecC* genes. In total 113 *S. aureus* positive samples were detected. Poultry was positive in 40% (32/80 meat and 8/12 liver), beef in 24% (16/53; 3/26) and pork in 35% (40/114; 14/41) of samples. In 4 (1.2%) samples of pork products (2 meat and 2 liver samples) MRSA were confirmed. All MRSA isolates were subjected to the ST398-specific PCR with positive results. This study shows that the prevalence of *S. aureus* in meat is high, however, the occurrence of MRSA is low in the Czech Republic compared to other European countries.

The results of the project LO1218 were obtained with a financial support from the MEYS of the CR under the NPU I program and MZCR NAZV KUS QJ1210284.

Board No: 69 *(New and Re-) Emerging Zoonotic Diseases*

GENERATION OF A *GIARDIA DUODENALIS* BIOBANK FOR FUNCTIONAL EPIDEMIOLOGY

C. Klotz[1], J. Hahn[2], R. Ignatius[3], P. Gosten-Heinrich[1], T. Aebischer[1]

[1]Department for Mycotic and Parasitic Agents and Mycobacteria, Robert Koch Institut, Berlin, Germany, [2]Institute of Pharmaceutical Biology, Freie Universität Berlin, Berlin, Germany, [3]Institute of Tropical Medicine and International Health, Charité – University Medicine Berlin, Berlin, Germany

Infections with the protozoan parasite *Giardia duodenalis* are a worldwide problem for Public Health. Up to 20% of giardiasis cases are treatment refractory to common therapy with nitroimidazoles. The underlying virulence factors of the disease are largely unknown. This project aims at the generation of a biobank and data base of *G. duodenalis* isolates from Germany as a tool for functional epidemiology. Trophozoite cell lines are established from purified *G. duodenalis* cysts by *in vitro* excystation and culture protocols. The parasites are genetically characterized by analyzing the genomic sequence of established marker genes. The established trophozoite cell lines are characterized in functional assays regarding drug susceptibility or potential virulence factors. Currently, 203 cysts samples were subjected to *in vitro* excystation and long-term trophozoite cultures could be established for 20 isolates (9%). Genetic characterization of the cysts revealed *G. duodenalis* type B in approximately 80% of the samples. In stark contrast, 90 % of the trophozoites from the established long-term cultures belonged to type A. This confirms that culture conditions preferentially support growth of type A isolates. Selected isolates have been characterized in more detail *in vitro* for their drug susceptibility to metronidazole and orlistat, a lipase inhibitor with potent *Giardia*growth inhibitory effects *in vitro*. The susceptibility to metronidazole and orlistat ranged between IC50 values of 5-13 µM and 1-7 µM, respectively, depending on the parasite isolate type. In conclusion, the long-term *in vitro* growth of 20 new *G. duodenalis* isolates reveals a starting point for functional analysis. In particular, drug susceptibility and the diversity of potential virulence factors will be analysed in future in more detail to correlate genetic traits with functionality. However, the generation of long-term *in vitro* cultures of *G. duodenalis* trophozoites remains challenging, in particular for type B parasites.

Board No: 70 *(New and Re-) Emerging Zoonotic Diseases*

FOSTERING BIOSAFETY AND BIOSECURITY IN A CHANGING WORLD - COMPONENT DETECTION AND DIAGNOSTIC INTRODUCES ITSELF

C. Kohl[1], D. Bourquain[1], J. Michel[1], C. Yue[1], C. Uhlenhaut[1,2], A. Nitsche[1]

[1]Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany, [2]Strengthening Global Biosecurity, Robert Koch Institute, Berlin, Germany

Some biological agents (e.g. viruses, bacteria, biotoxins) can pose a major threat to public health. They can be of natural origin (e.g. zoonotic transmission), accidental releases (biosafety issues) or intentional releases (e.g. bio terror attack). The Federal Republic of Germany offers the German Partnership Program for Excellence in Biological and Health Security to several countries around the world strengthen their capabilities for biological risk management. The Program was launched by the German Federal Foreign Office as contribution to the Global Partnership Against the Spread of Weapons and Materials of Mass Destruction.The comprehensive program is implemented by the German International Co-Operation (Gesellschaft für Internationale Zusammenarbeit, GIZ) and the Robert Koch Institute (RKI). It is complemented by three distinguished German institutions (Bernhard-Nocht-Institute for Tropical Medicine, the Friedrich-Loeffler-Institute for animal health and the Institute for Microbiology of the German Armed Forces). One of the main pillars of the program is the advancement of capacities and capabilities in the field of detection and diagnostics. Here, we describe expertise, support and cooperation offered by the RKI in this field.

Board No: 71 *Novel Methods and Diagnostics*

STUDIES ON THE REGULATION AND EXPRESSION OF MECC GENE-DETERMINED BETA-LACTAM RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

B. Ballhausen, A. Kriegeskorte, D. Kuhn, N. Schleimer, G. Peters, K. Becker

Institute of Medical Microbiology, University Hospital Münster, Münster, Germany

Recently, novel methicillin-resistant *Staphylococcus aureus* (MRSA) strains were discovered that carry the *mecC* gene instead of *mecA*. In *S. aureus*, methicillin resistance relies on a low-affinity penicillin-binding protein (PBP), designated PBP2a, which is usually encoded by *mecA*. *mecC*, which shares only 70% DNA homology to *mecA*, is embedded within the chromosomal cassette SCC*mec* XI (along with the regulatory elements *mecRI/mecI*). To date, the proof has still been missing that *mecC* builds the genetic basis for beta-lactam resistance in SCC*mec*XI-harboring *S. aureus* strains.
Here, a *mecC* gene knock-out in *S. aureus* with SCC*mec*XI background was generated and a direct comparison of *mecA* and *mecC* was carried out while expressing each gene in trans within different *S. aureus* strains (RN4220, ME131, NE1868 and w44646Δ*mecC*). Cefoxitin and oxacillin minimal inhibitory concentrations (MICs) were determined. Since comparatively low MICs for beta-lactam antibiotics were observed for SCC*mec*XI-harboring *S. aureus* and to unravel underlying molecular processes, the transcriptional promotor activity of *mecA* and *mecC* was examined.
The Δ*mecC* mutant exerted notably reduced cefoxitin and oxacillin MICs compared to the wild type strain. Thereby, we provided evidence that *mecC* and its gene product is conferring beta-lactam resistance. The expression of *mecC* led to an increase in MICs for oxacillin and cefoxitin in all strain backgrounds, similar to *mecA*. The gene expression of *mecC* as well as of *mecI* and *mecRI*(encoding for the regulatory system) in *S. aureus* with SCC*mec*XI background was inducible by oxacillin. The analyses revealed a higher activity for the promotor of *mecA* relative to that of *mecC*. The MecI regulatory protein (repressor molecule) and the corresponding binding site within the *mec* operator region showed remarkable differences between *mecA*- and *mecC*-harboring *S. aureus*, speculating that regulatory effects lead to variations in the resistance mechanism.
In summary, it could be proven that *mecC* is the resistance determinant in MRSA carrying the SCC*mec* XI. Likewise, *mecC* was shown to mediate oxacillin and cefoxitin resistance in different genetic *S. aureus* strain backgrounds. Overall, the study provided the missing molecular link between *mecC* and beta-lactam resistance in *S. aureus*.

Board No: 72 *Novel Methods and Diagnostics*

OBTAINING MONOCLONAL ANTIBODIES TO CARDIAC MARKERS I AND T

S.N. Borovikov, A.S. Syzdykova, G.O. Kartabaeva, A.N. Dzhangulova

Agricultiral Biotechnology Research Institute, Seifullin Kazakh Agro Technical University, Astana, Kazakhstan

Acute myocardial infarction (AMI) is one of the major causes of death in many countries. Medicine in recent years has made great strides in the treatment of this disease. However, the effectiveness of treatment depends on the time between the occurence of acute heart failure and the initiation of therapeutic measures.

Therefore, timely diagnosis plays a major role in the successful treatment. Rapid tests for the early diagnosis of AMI based on detection of blood cardiac markers (troponin I and T) are becoming more widespread in the clinic.

The aim of our work was to obtain specific monoclonal antibodies (mAbs) to biomarkers of myocardial injury, which can be used to develop rapid tests allowing to determine the components of myocardial infarction troponin complex of myocardiocytes, i.e. troponins I and T in the blood of patients suspected with acute myocardial infarction.

Immunization of mice Balb /c with preparations of cardiac troponin I and T was performed (maximum titer was 1:12800). Immune splenocytes were isolated, and hybridization with myeloma line cells X 63. Ag.8.6.5.3. was carried out by standard methods.

At the expiration of 7-10 days the formation of hybrid clones was observed. Culture fluid from the wells containing single clones was tested by indirect ELISA. As a result, 2 and 5 clones producing antibodies to cardiac preparations of Tropinin I, and Troponin T were selected, respectively.

According to the results of cloning Hybridomas most actively synthesizing antibodies to cardiac troponin I designated as 2H2C3, 2H2G9, 3C5H5, and clones 2B6G2, 2B6B3 and 2B6E7 producing antibodies to troponin T were selected for further research.

Monoclonal antibodies were accumulated in preparative amounts and their immunochemical properties were investigated. Isotypes of mAbs were defined and the binding constant indicators (affinity) with respect to used antigens were determined (0.85 × 10 8 M - 2.0 x 10-9M).

Thus, the strains of hybrid cells were obtained that produce monoclonal antibodies specific for different epitopes of cardiac troponin I and T. The results of studying immunochemical and immunobiological properties of mAbs suggest the possibility of using these antibodies in the development of rapid diagnostic tests for the diagnosis of myocardial infarction.

Board No: 73 *Novel Methods and Diagnostics*

NOVEL APPROACH TO EVALUATE EFFICACY OF PROBIOTIC ISOLATES AGAINST ENTERIC PATHOGENS IN ANIMALS

K. Arsi[1], A. Donoghue[2], A. Woo-ming[1], P. Blore[1], D. Donoghue[1,2]

[1]Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, United States, [2]PPPSRU, USDA, Fayetteville, United States

Campylobacter is an enteric pathogen and a leading cause of foodborne illness worldwide, primarily caused by consumption of contaminated poultry products. Many strategies have been tried to eliminate Campylobacter from live poultry with limited success. One strategy to reduce Campylobacter colonization in poultry is by the use of oral probiotics. Probiotics are live microorganisms which when administered in adequate amounts can potentially eliminate enteric pathogens. Unfortunately, oral probiotics produce variable results, possibly due to destruction in the acidic stomach. Encapsulation of isolates may overcome this problem but there is no assurance these isolates will have efficacy in the lower GI tract. Therefore, screening candidate bacterial isolates by directly placing them in the lower intestinal tract (bacterial transplantation) may eliminate the time and expense of encapsulating ineffective isolates. This strategy of transplanting bacterial isolates (fecal transplantation) has been successfully used in humans to eliminate the enteric pathogen, clostridium difficile. Therefore, the purpose of this study was to collect bacterial isolates with anti-Campylobacter activity and evaluate their efficacy *in vivo* upon either oral or lower gastrointestinal administration. Bacterial isolates were collected from healthy bird sand screened for anti-Campylobacter activity *in vitro* by using a soft agar overlay technique. Ten isolates demonstrating anti-Campylobacter activity were either orally gavaged or administered in the lower intestine (intra-cloacally) of chicks (n=10/isolate/location, total 200 chicks) on day 1 of age at a dose of 1x107CFU per individual isolate. On day 7, all the birds were orally gavaged with a mixture of wild type Campylobacter (1x107 CFU). On day 14, birds were euthanized and ceca contents were enumerated for Campylobacter counts. When isolates were dosed orally, only one isolate showed a 1 log reduction in cecal Campylobacter counts whereas when administered in the lower intestine, five of these isolates produced a 1-3 log reduction in cecal Campylobacter counts. Thus, bypassing the acidic environment of the stomach apparently allows more isolates to survive and effectively reduce Campylobacter colonization. These results support the strategy of evaluating the efficacy of probiotic isolates via cloacal inoculation prior to undergoing the effort of protecting isolates (e.g., encapsulation) for oral administration.

Board No: 74 *Novel Methods and Diagnostics*

DETECTION AND DIFFERENTIATION OF BOTULINUM NEUROTOXIN SEROTYPES C AND D BY MASS SPECTROMETRY-BASED ENDOPEPTIDASE ASSAY

E.M. Hansbauer[1], M. Skiba[1], T. Schreiber[1], S. Sonntag[1], J. Wolf[1], F. Finkenwirth[1], M.B. Dorner[1], A. Rummel[2], B.G. Dorner[1]

[1]Centre for Biological Threats and Special Pathogens Zbs3, Robert Koch Institute, Berlin, Germany, [2]Institut Für Toxikologie, Medizinische Hochschule Hannover, Hannover, Germany

Botulinum neurotoxins (BoNT) produced by Clostridium botulinum are known as causative agents of botulism, a severe paralytic illness in men and animals. Whereas BoNT serotypes A, B, E and F are associated with human disease, BoNT/C and /D cause botulism in animals. Cases of botulism due to serotypes C and D have worldwide distribution in animal husbandry and wildlife. While serotype C is prevalent in avian botulism, both serotypes C and D are associated with botulism in cattle. The detection and differentiation of BoNT/C and D has been challenging based on the known sequence homology (up to 74% amino sequence identity) and the existence of mosaic isoforms. The toxin can be present and functionally active in the absence of the producing organism and its genetic information, calling for highly sensitive and specific protein detection methods. In this work, we developed a sensitive mass spectrometry-based assay to detect and differentiate BoNT/C and /D using its highly specific endoprotease activity.

Board No: 75 *Novel Methods and Diagnostics*

CYTOTOXICITY AND ANTI FMD EFFICACY OF ARIA-ORAL® HERBAL COMPOUND

J. Kaboutari katadj[1], A. Dabili[2], S.H. Dehkordi[1]

[1]Basic Sciences, Shahrekord University, Shahrekord, Islamic Republic of Iran, [2]Physiology, Pharmacology & Toxicology, University of Tehran, Tehran, Islamic Republic of Iran

Keywords: FMD, Aria-oral®, cytotoxicity, antivirus.

Introduction: FMD is a viral disease which imposed heavy economic losses to animal husbandry around the world. Since attention toward natural remedies has been increased in recent years, the aim of this study was to *in vitro* evaluation of cytotoxicity and antiviral properties of a novel herbal compound Aria-oral®which is composed of Eugenol, Rebarb in Acetic acid and apple vinegar against FMD virus of O panasia strain.
Material & Method: Serial dilutions of 0.128 to 0.00006% for cytotoxicity and 0.000125%,0.0000625% &0.000003%of Aria-Oral®added to 105TCID50/ml of FMD virus Opanasia strain with 2,3&4 hours of exposure time in 96 holes plates for antiviral evaluation were made. Experiments were done 2 times each with 3 replicates. IBR-S2 cell line and Neutral Red Uptake assay after 24 hours of incubation at 370c were used and optical density at 540nm was read. Comparison between average of 6 replicates was done by one way ANOVA and Duncan test ($P<0.05$).
Results: The highest safe concentration of Aria-oral® with the least toxicity was 0.000625%. Antiviral property was seen with 0.000003% and 0.0000625% concentration of Aria-oral® after 3&4 hours of exposure, but after 4 hours of exposure time, antiviral property was significantly more prominent.
Conclusion: Aral-oral® can be assumed as a promising herbal anti FMD compound with suitable toxicity and time dependent antiviral property.

Board No: 76 *Novel Methods and Diagnostics*

NEW EPITOPES FOR SEROLOGICAL DENGUE VIRUS DIAGNOSTICS

A. Kerzhner[1], L. Barzon[2], M. Niedrig[3], S. Ulbert[1]

[1]Department of Immunology, Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany, [2]Department of Molecular Medicine, University of Padova, Padova, Italy, [3]Centre for Biological Security, Robert Koch Institute, Berlin, Germany

Dengue Virus (DENV) is a mosquito borne human pathogen which belongs to the family of flaviviruses and causes over 100 million infections annually. The DENV serocomplex consists of four (DENV 1-4) coexisting serotypes. A Specific, inexpensive and easy-to-use laboratory diagnosis is essential to confirm DENV infections, the early inducement of appropriate treatment and epidemics control. Current laboratory serological DENV tests lack specificity due to cross-reactivity of antibodies from other flavivirus infections such as West Nile Virus (WNV), Yellow Fever Virus (YF) or Tick Borne Encephalitis Virus (TBEV). The E-(envelope) protein is a major target of the humoral immune response against flaviviruses, and is mostly used as antigen for IgM- or IgG- based ELISAs. However, due to the conserved nature of some of its domains, it also binds the majority of cross-reactive antibodies. Therefore new specific targets have to be considered for dengue diagnostics. For this purpose, antibody responses to human dengue infection have been measured. The three structural proteins and the non structural protein 1 (NS1) of DENV-2 were portioned into 60 overlapping 30mer peptides and fused to a GST tag. After the expression in *E.coli* and purification through glutathione affinity and size exclusion chromatography, the proteins were screened in IgG based ELISAs for new epitopes that bind antibodies of DENV infected, but not WNV-or TBEV infected human sera. Several peptides showed significant antibody response in the region of E- and NS1 protein to DENV infected sera and were selected from the first screening. Those are currently being analyzed for the detection and possible discrimination of infections with the remaining DENV serotypes. The findings could be used for the development of a specific serological DENV assay.

Board No: 77 *Novel Methods and Diagnostics*

COMPARISON OF COMMERCIAL RNA AND DNA EXTRACTION KITS FOR THE RECOVERY OF DIFFERENT VIRUSES BY NEXT GENERATION SEQUENCING (NGS)

J. Klenner, C. Kohl, A. Radonić, P.W. Dabrowski, A. Nitsche

Centre for Biological Threats and Special Pathogens, Zbs 1, Highly Pathogenic Viruses, Robert Koch Institute, Berlin, Germany

A crucial step in the detection of viruses in clinical samples is the efficient extraction of nucleic acids. Limiting factors can be the sample volume and the virus concentration, therefore it is critical to achieve a high yield of nucleic acids. While specific approaches (i.e. real-time PCR) are highly sensitive but usually limited by their respective target, the open view of NGS is challenging because the ensuing bioinformatics analysis determines the sensitivity.

In this study five commercial QIAGEN kits (Viral RNA Mini Kit, DNA Blood Mini Kit, cador Pathogen Mini Kit, UltraSens Virus Kit and MinElute Virus Spin Kit) were evaluated by NGS, comparing the nucleic acid yields and read output numbers for four different model viruses (Reovirus, Sendai virus, Influenza virus and Vaccinia virus), each at defined concentrations in the same sample. Total nucleic acid yield was divided into DNA and RNA, respectively. RNA samples were subjected to DNAse digestion by Turbo DNA-free (Ambion) and transcribed into cDNA before second-strand synthesis. Prior to NGS, the yield of nucleic acids was determined by NanoDrop, Qubit and quantitative real-time PCR. Libraries were prepared for Illumina sequencing on a HiSeq 1500 system in rapid-run mode. A paired-end protocol was used with read lengths of 2 x 150 bp. The bioinformatics analysis was performed by mapping the obtained reads to the known genome sequences of the four viruses.

As presented here, evaluation of the different commercial extraction kits indicates differences in the numbers for RNA and DNA reads, depending on the kits used.

Board No: 78 *Novel Methods and Diagnostics*

MONOCLONAL ANTIBODIES FOR FUNCTIONAL ANALYSES OF BOTULINUM NEUROTOXIN COMPLEX PROTEINS IN INTESTINAL RESORPTION

M. Krüger[1], D. Stern[1], S. Worbs[1], M.B. Dorner[1], A. Rummel[2], B.G. Dorner[1]

[1]Centre for Biological Threats and Special Pathogens, Biological Toxins, Robert Koch Institut, Berlin, Germany, [2]Institute for Toxicology, Hannover Medical School, Hannover, Germany

Botulinum NeuroToxins (BoNTs) are produced by *Clostridium botulinum* and different other Clostridia (*C. butyricum, C. baratii, C. argentinense*) and represent the most lethal substances known so far. In Germany, clinical relevant intoxications are primarily caused by ingestion of contaminated food. To reach their target location at neuromuscular junctions triggering flaccid paralysis, BoNTs have to pass the intestinal barrier by a yet unidentified mechanism.

The neurotoxins form complexes with different non-toxic accessory proteins. NTNH (non-toxic non-hemagglutinin) is considered to protect the neurotoxin from proteolytic digestion and hydrolysis in the acid environment of the gastrointestinal tract. The role of several associated hemagglutinins (HA33, HA17, HA70) is less clear at present, for HA33 there is indication for a role in uptake in the intestine. In some BoNT-producing strains, HA proteins are missing and are replaced by several OrfX proteins; for OrfX proteins, the expression and physiological role is even less defined.

In order to analyse the functional role of BoNT complex proteins in detail, we aim at setting up a mouse model mimicking oral intoxication. As a first step, we have generated monoclonal antibodies (mAb) against different BoNT complex proteins (hemagglutinins, NTNH and OrfX). Here we report on advanced immunisation and screening strategies to identify a panel of high affinity mAb recognizing multiple epitopes on BoNT complex proteins.

Board No: 79 *Novel Methods and Diagnostics*

CLONING STRATEGIES FOR UNSTABLE INFLUENZA HEMAGGLUTININ SEGMENTS: TOWARDS RAPID VACCINE PRODUCTION

A. Mostafa[1,2], P. Kanrai[1], H. Petersen[3], S. Ibrahim[4], S. Rautenschlein[3], J. Ziebuhr[1], S. Pleschka[1]

[1]Institute of Medical Virology, Justus Liebig University Giessen, Giessen, Germany, [2]Center of Scientific Excellence for Influenza Viruses, National Research Center (NRC), Giza, Egypt, [3]Clinic for Poultry, University of Veterinary Medicine Hannover, Hannover, Germany, [4]Department of Genetic Engineering, Veterinary Serum and Vaccines Research Institute (VSVRI), Agricultural Research Center (ARC), Cairo, Egypt

Influenza A viruses (IAVs) are the most relevant and continual source of sever infectious respiratory complications in humans and poultry. Therefore, an efficient vaccination that elucidates protective and neutralizing antibodies against the viral hemagglutinin (HA) and neuraminidase (NA) is an important strategy to face and control annual epidemics or occasional pandemics caused by emerging IAVs. With the help of plasmid-based reverse genetics technology, it is possible that candidate vaccine seed virus (CVSV) are rapidly generated by combination of newly cloned HA and NA cDNAs from IAV of concern with the plasmids encoding the other viral segments of a high-yield donor virus to prepare either inactivated or live attenuated vaccines within few weeks after the isolation of the circulating epidemic or pandemic IAV. However, the correct and high quality production of the plasmids encoding the cDNAs corresponding to the HA and NA segments in bacterial cells is an essential step for the successful and efficient rescue of the CVSV by recombinant DNA technology. The instability of some HA-cDNAs cloned and transformed into competent bacteria represents a major obstacle. Here we offer a solution for this problem as demonstrated by efficient cloning of different unstable HA segments (H5- and H9-subtypes) employing a newly constructed vector for reverse genetics (pMKP*ccdB*) and an specific *Escherichia coli* strain.

Board No: 80 *Novel Methods and Diagnostics*

OPTIMIZATION OF BOTULINUM NEUROTOXIN SYNAPTIC PROTEIN CLEAVAGE BY THE TAGUCHI DESIGN-OF-EXPERIMENT METHOD

L. Reckzeh[1], D. Stern[1], A. Rummel[2], M. Dorner[1], B. Dorner[1]

[1]Centre for Biological Threats and Special Pathogens Zbs3, Robert Koch Institute, Berlin, Germany, [2]Institute for Toxicology, Hannover Medical School, Hannover, Germany

Botulinum neurotoxins (BoNTs) cause the rare, but life-threatening disease botulism by cleaving proteins of the synaptic SNARE-complex, thereby blocking neurotransmitter release at neuro-muscular junctions and inducing flaccid paralysis.
BoNT detection is highly challenging since so far eight serotypes with more than 40 subtypes have been described which differ on amino acid level up to 36%. As 'gold-standard' for the detection of all BoNT molecules in clinical and food samples, the mouse bioassay is still commonly used which is ethically questionable. Numerous attempts to develop alternative detection strategies have been made in the past. Still, an *in vitro* assay enabling the detection of all BoNT sero- and subtypes with acceptable sensitivity and specificity is not yet available.
A major requirement for assay development is the establishment of proper cleavage conditions for the different BoNT molecules, as buffer composition and the addition of supplements such as stabilising proteins, ion strenght and detergents tremendously influence the catalytic activity of BoNT. In this work, we systematically analysed different cleavage conditions using the Taguchi method in order to find optimal requirements with maximum substrate cleavage for each BoNT serotype. Furthermore, we aimed at developing a 'standard buffer recipe' that can be applied to all serotypes in order to establish a highly sensitive functional multiplex approach for BoNT molecules.

Board No: 81 *Novel Methods and Diagnostics*

COMPARISON OF SAMPLE PREPARATION METHODS FOR THE RELIABLE DETECTION OF ZOONOTIC PATHOGENS IN FOOD

A. Rohde, J.A. Hammerl, B. Appel, R. Dieckmann, S. Al Dahouk

Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

The identification of bacterial pathogens in food is still largely performed by cultivation on adequate growth media following widely accepted procedures such as ISO and DIN standards. For the successful recovery of the target bacteria out of a food matrix in a viable and replication-competent state, sampling plans and different homogenization tools have been developed. The majority of these devices rely on the application of mechanical forces with varying magnitude. However, comprehensive data comparing these systems is scarce. Thus, we systematically investigated the effect of sample preparation by examining *Salmonella enterica* spiked meat products.
Four different homogenization methods (Stomacher, Fastprep24, Speedmill or ultrasonic devices) were investigated for different meat products, which were either contaminated on the surface of chicken breast or within the food matrix (i.e. various kinds of sausages made of pork spiked with different numbers of *S. enterica* during production). In addition, the distribution of the microbial burden within the sausages was examined by comparing different sampling regions (a) the inner core, (b) the outer rim and (c) whole cross-sections of the sausages. For surface contaminations all four homogenization methods showed comparable results and only minor differences regarding the detection of the target pathogen by cultivation after ISO standards and direct plating of homogenates. In contrast to that, in case of sausages with pathogens located within the food matrix, the Fastprep24 system, considered as a relatively harsh homogenization technique, showed significantly better performances compared to sonication or stomaching.
In conclusion, the high importance of an optimized sample preparation for efficient monitoring of food products becomes apparent since the choice of the homogenization device as well as the sampling procedure have substantial impact on subsequent analyses. Especially for food samples where pathogens are located predominantly within the matrix or with a pathogen load near the detection limit, care should be taken in the selection of the homogenization technique. The results are not only useful for culture-based approaches, but can also serve as reference point for direct whole-cell detection methods which are independent of cultivation, for example fluorescence *in situ* hybridization (FISH).

Board No: 82 *Novel Methods and Diagnostics*

IMPROVED MOLECULAR DETECTION OF *TOXOPLASMA GONDII* OOCYSTS IN ENVIRONMENTAL SAMPLES

B. Prewein[1,2], R. Kunert[2], F. Allerberger[1], W. Ruppitsch[1,2]

[1]Institute of Medical Microbiology and Hygiene, Austrian Agency for Health and Food Safety, Vienna, Austria, [2]Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Objectives: *Toxoplasma gondii* is a protozoan parasite, which reproduces sexually in felids. Thereby, oocysts are formed and released to the environment, including soil, vegetables, fruits and water. Uptake by humans can lead to toxoplasmosis, an illness with severe consequences for immunocompromised patients and foetuses. The aim of our study was the evaluation and development of a robust and sensitive technique for accurate detection of oocysts in environmental samples.
Methods: Various pre-treatments based on temperature alterations and mechanical forces were evaluated. For determining the optimal DNA extraction method, several kits suitable for isolation of high-quality DNA from difficult samples from diverse companies were tested. Different primer sets and TaqMan® probes specific for the B1 gene and AF 487550 gene were evaluated for specific detection of *T. gondii*.
Results: The optimal pre-treatment of *T. gondii* oocysts prior to DNA isolation were repeated freezing and thawing cycles with -80°C and 25°C with a freezing rate of approximately 1°C/min. Twenty-nine times as much DNA could be detected compared to no pre-treatment. The Epicentre QuickExtract™ Plant Solution was defined as the best DNA isolation technique. Forty-two times as much DNA could be detected compared to the standard Qiagen QIAamp® DNA mini kit. One inhouse designed and a published primer set with modified TaqMan® probe displayed the best PCR results for *T. gondii* detection compared to current methods. Combination of best pre-treatment and extraction method resulted in an over 1000 times increase of detectable DNA.
Conclusions: This was the first study showing that oocyst pre-treatment with a slow freezing rate, the use of DNA extraction kits without columns and the modification of TaqMan® probes improve the detection of *T. gondii* oocysts by a factor of over 1000. It indicates the importance of pre-treatment and the limitations of conventional DNA extraction methods.

Board No: 83 *Novel Methods and Diagnostics*

METHOD FOR HEPATITIS E VIRUS DETECTION IN RAW SAUSAGES AND LIVER SAUSAGES FROM DOMESTIC PIGS

K. Szabo[1,2], E. Trojnar[1], A. Binder[3], H. Anheyer-Behmenburg[3,2], U. Schotte[3], L. Ellerbroek[1], G. Klein[2], R. Johne[1]

[1]Federal Institute for Risk Assessment, Unit Food Hygiene and Virology, Berlin, Germany, [2]University of Veterinary Medicine Hannover, Institute of Food Quality and Food Safety, Hannover, Germany, [3]Central Institute of the Bundeswehr Medical Service, Department of Veterinary Medicine, Kiel, Germany

The number of cases of human hepatitis E in Germany has constantly increased in the recent years. In 2013, a total of 458 cases of illness were reported to the Robert Koch Institute. Beside infections, which were imported from endemic regions of Africa and Asia, a zoonotic and foodborne transmission of hepatitis E virus (HEV) through consumption of raw and undercooked liver, meat, or sausages from domestic pigs, wild boar, and deer has been documented. However, investigation of suspect food samples in the context of disease outbreaks is hampered by the lack of sensitive and reliable methods for HEV detection in involved food matrices.

The aim of this study was to develop a sensitive and reliable technique for the detection of HEV in raw sausages and liver sausages from domestic pigs. For this purpose, a number of different methods for purification and concentration of HEV were tested and optimized using artificially HEV-contaminated pig sausage samples. The detection of viral RNA was performed by real-time RT -PCR.

The best HEV detection rates for raw pig sausage could be achieved with a direct RNA extraction method using TRI Reagent® Solution. The recovery rates ranged from 8% to 25 % (M = 11, SD = 4). The detection limit of the method was 7.0×10^3 genome equivalents per g raw sausage. Application of this method to liver sausage resulted in a considerable higher detection limit. For this reason, an adaptation of the technique to the respective matrix is currently performed. However, using this method we were able to detect HEV in one out of five liver sausages purchased from retail. It can be concluded that the presented method is a reliable method for the detection of HEV in raw sausages and liver sausages from domestic pigs which may be used for analysis of the distribution of HEV in retail sausages as well as during outbreak investigations.

Board No: 84 *Novel Methods and Diagnostics*

INFLUENZA A VIRUS ENCODING SECRETED GAUSSIA LUCIFERASE AS USEFUL TOOL TO ANALYZE VIRAL REPLICATION AND ITS INHIBITION BY ANTIVIRAL COMPOUNDS AND CELLULAR PROTEINS

N. Eckert, F. Wrensch, S. Gärtner, N. Palanisamy, U. Goedecke, N. Jäger, S. Pöhlmann, M. Winkler

Infection Biology, German Primate Center, Göttingen, Germany

Employing a previously described design, we have generated several replication-competent influenza A viruses carrying either fluorescent proteins or Gaussia luciferase, which is secreted into the medium. Infection with the viruses encoding fluorescent proteins was readily detectable, but the virus encoding Gaussia luciferase was more stable during repeated cell-culture passage and was therefore analyzed in detail. Quantification of Gaussia luciferase activity in the supernatants of infected cultures allowed the convenient and highly sensitive detection of viral spread, and enzymatic activity correlated with the number of infectious particles released from infected cells. Furthermore, the Gaussia luciferase encoding virus allowed the sensitive quantification of the antiviral activity of the neuraminidase inhibitor (NAI) zanamivir and the host cell interferon-inducible transmembrane (IFITM) proteins 1–3, which are known to inhibit influenza virus entry. This shows that the Gaussia luciferase encoding virus is a highly useful tool for screening of antiviral compounds and the characterization of cellular proteins with antiviral activity. Finally, the Gaussia luciferase encoding virus was used to demonstrate that influenza A virus infection is sensitive to a modulator of endosomal cholesterol, in keeping with the concept that IFITMs inhibit viral entry by altering cholesterol levels in the endosomal membrane. In sum, we report the characterization of a novel influenza A reporter virus, which allows fast and sensitive detection of viral spread and its inhibition, and we show that influenza A virus entry is sensitive to alterations of endosomal cholesterol levels.

Board No: 85 *Ecology of Emerging Zoonoses*

AGE, HOSPITALIZATION TYPE AND SEX PREVALENCE OF *E. COLI* O26&O91 STRAINS ISOLATED FROM DIARRHEAL CASES OF SHAHREKORD & BOROUJEN TOWNSHIPS

J. Kaboutari katadj[1], S.H. Dehkordi[1], J. Cheraghi[2]

[1]Basic Sciences, Shahrekord University, Shahrekord, Islamic Republic of Iran, [2]Basic Sciences, Ilam University, Ilam, Islamic Republic of Iran

Keywords: E. coli, Diarrhea, age, hospitalization

Introduction: *E. coli* is one the most important pathogens of diarrhea of human and animal species. This study was conducted to survey the relationship between prevalence of *E. coli* O26&O91 strains isolated from diarrheal cases from Shahrekord and Boroujen Townships in Iran based on hospitalization type, sex and age of patients.
Martial &method: 284 diarrheal samples were taking from Shahrekord and Boroujen Townships hospitals and by complementary bacteriological tests and PCR, 30 *E. coli* positive cases were isolated and then after they were categorized based on the age (2-15, 16-24 & 25-60 years), hospitalization type (in patient& outpatient) and sexuality of patients. Data were analyzed by using Chi square, Fisher exact and Multi regression Logistic statistical
Results: The results shown that prevalence of positive cases were 49.6% and 50.4% for male and female, 72.5% outpatients and 27.5% for inpatients. Prevalence of isolated cases was 51.9%, 31.3% and 16.8% for 2-15, 16-24 and 25 to 60 years of old respectively.
Conclusion: The prevalence of *E. coli* isolates was not different between male and female, but the most prevalent cases were belong to outpatients and 2-15 years old which shows the importance of *E. coli* and needs to more attention in this groups.

Board No: 86 *Ecology of Emerging Zoonoses*

ECOLOGY AND GENETIC STRUCTURE OF *BORRELIA BURGDORFERI S.L.* IN DIVERSE HABITATS OF SLOVAKIA

M. Derdáková[1], D. Selyemova[1], R. Vaclav[1], M. Chvostac[1], S. Kolencik[2], K. Cabadajova[2], E. Ondriskova[3], T. Vaculova[1], V. Taragelova[1]

[1]Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Faculty of Natural Sciences, Commenius University, Bratislava, Slovakia, [3]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Over the past decades, *Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis, has attracted a lot of scientific attention. At the time of its discovery, it was thought to be an uniform organism. Currently 19 different genospecies belong to this complex out of which at least 9 are present in Europe. Prevalence in ticks in Slovakia varies between 10 to 40%. The specific associations of different genospecies with the reservoir hosts as well as clinical symptomatics have been assigned. This association is not strict and difference have been observed. The incidence of LB in Slovakia is up to 20 cases per 100 000 inhabitants.

From 2002 till 2013 we have analyzed prevalence, genetic variability and ecological associations of *B. burgdorferi* s.l. in different habitats of Slovakia. 6024 questing Ixodes ricinus ticks were sampled from 10 diverse habitats including mountain spruce forest, lowland deciduous forest, xerothermic steppe, suburban forest, urban park, game reserve and woodland-farmland ecotone. Moreover, at two sites (urban and sylvatic) host feeding ticks collected from birds, lizzards and rodents were analyzed as well.

18.7% ticks were positive for *B. burgdorferi* s.l. There was significant difference in prevalences between the habitats. The lowest prevalence (6%) was in urban park in 2013, the highest prevalence - 46% was detected in 2010 at site in foothill area of Central Slovakia. Moreover, we observed significant differences in prevalences between studied years . We have detected 7 genospecies occurring in Europe. *B. afzelii, B. garinii* and *B. valaisiana* were detected at each studied site as the most prevalent with the few exceptions. RFLP method revealed the presence of two different genotypes, that was confirmed by phylogenetic analyses. Using the SSCP method we were able to distinguish *B. bavariensis* from other *B. garinii* genotypes. In habitats with higher biodiversity and abundance of hosts – natural lowland forests - the most commonly detected genospecies were *B. afzelii, B. garinii* and *B. valaisiana.*

In habitats with lower biodiversity and abundance of hosts –the genospecies that are not so common for this region dominated or were present (*B. lusitaniae* –mountains; *B. spielmanii*, *B. burgdorferi* s.s.-urban park). This might indicate that introduction of new genospecies migh be more easily established in the areas without presence or with low occurence of originall-native genospecies represented by the lower abundance and diversity of reservoir hosts.

The study was supported by the project APVV-0267-10.

Board No: 87 *Ecology of Emerging Zoonoses*

AN ELEVEN YEARS STUDY OF THE PREVALENCE OF WEST NILE VIRUS NEUTRALIZING ANTIBODIES IN COMMON COOTS (*FULICA ATRA*) IN RELATION TO ENVIRONMENTAL CONDITIONS IN SOUTHERN SPAIN

J. Figuerola[1], F. Llorente[2], S. Ruiz[3], E. Pérez-Ramírez[2], J. Martínez-de la Puente[1], M. del Carmen Barbero[2], R. Soriguer[4], M.A. Jiménez-Clavero[2]

[1]Wetland Ecology, EBD CSIC, Sevilla, Spain, [2]CISA, INIA, Valdeolmos, Spain, [3]Servicio De Control De Mosquitos, Diputación De Huelva, Huelva, Spain, [4]Biodiversity Conservation, EBD CSIC, Sevila, Spain

West Nile virus (WNV) may be considered now an endemic virus in different countries in southern Europe. In Spain, regular evidence of WNV circulation has accumulated in the last decade. Common coots were used to monitor the seroprevalence of WNV neutralizing antibodies from 2003 to 2013 in Doñana (southern Spain). Serum samples were first tested by ELISA and positive and doubtful samples were further tested by seroneutralization against WNV and, in many of the cases, USUTU. To control for seasonal variation in antibody prevalence only samples of birds captured between October and November were analysed in this study (n=1101). Only two individuals presented higher neutralization titres for USUTU than for WNV. Prevalence of antibodies varied annually between 0 and 43% in first year birds and 0 and 82% in older birds. No clear temporal trend in antibody prevalence occurs and antibody prevalence has widely fluctuated across the 11 years of the study. Univariate correlations suggest that prevalence of antibodies in first year birds was positively related to the abundance of mosquitoes during the previous spring-autumn (r=0.74) but especially to the abundance of mosquitoes of the genus *Culex* during this period (r=0.80). Seroprevalence was also positively related to mean and minimum temperatures through the year (r=0.71). This positive relationship with temperature may be related to higher abundance of mosquitoes during the spring-autumn but also by better environmental conditions for mosquito (and virus) survival during the winter. The information available up to now suggests that the prevalence of antibodies was not particularly high in the years with WNV infection cases in humans and/or horses in Southern Spain. Consequently, identifying not only the factors related with WNV seroprevalence in wildlife, but also the processes that increase the contact rate of WNV infected mosquitoes with humans is necessary to understand the risk of WNV zoonotic outbreaks.

Board No: 88 *Ecology of Emerging Zoonoses*

CHARACTERIZATION AND DYNAMICS OF *SIPHONAPTHERA* POPULATIONS AND IMPACT ON HEALTH

E.B. Madoui[1], A. Bouattour[2], Z. Bouslama[1]

[1]Biology, "Emerging Diseases and Environment", Badji Mokhtar University, Annaba, Algeria, [2]Parasitology and Entomology, Pasteur Institute, Tunis, Tunisia

Keywords: *Siphonaptera* – fleas - infectious Diseases - PCR.

The history of biological associations may be observed as such associations predator-prey, host-parasite, host-symbiont The host-parasite systems in particular are one of the models for the study of evolutionary ecology copies. And parasitism is a way interspecific interaction defined by the exploitation of the living by the living (Combes 2001).
Parasitism is the most common way of life on this planet, involving representatives of major taxa, from the simplest single-celled organisms to complex vertebrates. Each species is potentially a victim of several parasites and, consequently, the number of parasite species greatly exceeds the number of species "autonomous" (Morel 1974).
So we are interested Flea, specifically pets (dog, cat and rat urban) which will be a more detailed study. The objective of this work is to identify the species found and highlight their role as vectors of pathogens, as well as about the importance of vector-borne diseases which are associated in our region (detection agents pathogens by PCR).

Board No: 89 *Ecology of Emerging Zoonoses*

IDENTIFYING THE LAST SUPPER: FEEDING PATTERN OF THE INVASIVE ASIAN MOSQUITO TIGER *AEDES ALBOPICTUS* AND THE NATIVE *CULEX PIPIENS* IN SOUTH EUROPE

J. Martínez-de la Puente[1], G. Capelli[2], A. Rizzoli[3], C. Aranda[4], T. Montalvo[5], J. Muñoz[1], R. Soriguer[6], J. Figuerola[1]

[1]Ecología De Humedales, Estación Biológica De Doñana (Ebd Csic), Sevilla, Spain, [2]Diagnostica Specialistica E Istopatologia, Istituto Zooprofilattico Sperimentale Delle Venezie, Legnaro, Italy, [3]Biodiversity and Molecular Ecology, Fondazione E. Mach, Trento, Italy, [4]Servei De Control De Mosquits, Consell Comarcal Del Baix Llobregat, Barcelona, Spain, [5]Servei De Vigilància I Control De Plagues Urbanes, Agència De Salut Pública De Barcelona, Barcelona, Spain, [6]Etología Y Conservación De La Biodiversidad, Estación Biológica De Doñana (Ebd Csic), Sevilla, Spain

Establishment of exotic mosquito vectors to new areas may create novel epidemiological scenarios with potential dramatic consequences together with autochthonous vectors like *Culex pipiens.Aedes albopictus* is currently expanding its distribution range to different continents. In particular, in Europe it was first recorded in 1979. *Ae. albopictus* has spread to countries of the Mediterranean region and north Europe where it has been incriminated in the transmission of both introduced (i.e. Chikungunya or dengue virus) and endemic (i.e. *Dirofilaria* nematodes) pathogens. *Culex pipiens* is the most abundant vector of West Nile virus and spread everywhere. Here, we applied a molecular approach to identify to the species level the blood meal source of engorged *Ae. albopictus* and *Cx. pipiens* collected from rural and urban areas from Italy and Spain during 2009 and 2011-2013. From 106 identified *Ae. albopictus* blood meals, mammals (97%) were by far the most common hosts found. Humans (92%) were the most common species in mosquitoes trapped in urban areas while rats (61%) dominated in rural areas. By contrast, from the 847 blood meals from *Cx. pipiens* identified, 654 (77%) had an avian origin and only 35 (4%) derived from humans. Overall, these results highlight the differential feeding pattern of both mosquito species and highly support the anthropophilic behaviour of *Ae. albopictus* in urban areas which may have important consequences for the transmission of introduced pathogens in Europe such as Chikungunya and Dengue virus. In comparison, due to its highly ornitophilic feeding behaviour *Cx. pipiens* may be more relevant for the transmission of virus that have on birds its main reservoirs (i.e. West Nile and USUTU virus).

Board No: 90 *Ecology of Emerging Zoonoses*

RECENT INCREASE OF DOBRAVA-BELGRADE VIRUS (DOBV) IN YELLOW-NECKED MICE IN NORTHERN ITALY

V. Tagliapietra[1], A. Rizzoli[1], R. Rosà[1], H. Hauffe[1], G. Marini[1], L. Voutilainen[2,3], T. Sironen[2], H. Henttonen[3]

[1]Department of Biodiversity and Molecular Ecology, Fondazione Edmund Mach (FEM), San Michele All'adige (TN), Italy, [2]Department of Virology, University of Helsinki, Helsinki, Finland, [3]Finnish Forest, Research Institute, Vantaa, Finland

Dobrava-Belgrade virus (DOBV) is the most pathogenic hantavirus in Europe with a case-fatality rate of up to 12%. DOBV infections have been reported in Italy following cross-sectional sero-epidemiological studies in rodents and humans since 2000, however, no clinical human cases have been confirmed thus far. In this study, we present a long-term pattern of serological changes in DOBV antibody prevalence in a population of A. flavicollis in the Province of Trento (northern Italy). From 2000 to 2012 the mean hantavirus seroprevalence was 2.7% (s.e.=0.3 %), ranging from 0% (in 2000, 2002 and 2003) to 12.5% (in 2012), a statistically significant increase (t-value=5.93, $p<0.001$). Using Generalized Linear Models (GLM) and multi-model inference, our results showed that the rise in mean annual precipitation together with higher individual body mass are responsible of the observed change in DOBV seroprevalence. We hypothesize that greater precipitation leads to a higher probability of survival and consequently, populations with a higher proportion of older (i.e. heavier) individuals, which have a higher rate of viral shedding. Alternatively, or in addition, increased precipitation could favour virus survival in the environment. Our findings underline the importance of a closer examination of these aspects and investigations are ongoing.

This study was partially funded by EU grant FP7 261504 EDENext and is catalogued by the EDENext Steering Commitee as EDENext222 (www.edenext.eu).

Board No: 91 *Ecology of Emerging Zoonoses*

IDENTIFICATION OF POTENTIAL MOSQUITOS VECTORS OF FLAVIVIRUS IN MIDDLE EBRO VALLEY, SPAIN

R. Pinal[1], J. Lucientes[1], L. Herrero[2], S. Delacour-Estrella[1], I. Ruiz-Arrondo[1], P.M. Alarcón-Elbal[1], M. Ana[1], M.P. Sánchez-Seco[2], A. Vázquez[2]

[1]Departamento De Patología Animal, Universidad De Zaragoza, Zaragoza, Spain, [2]Laboratory of Arbovirus and Viral Infectious Diseases, Institute of Health Carlos Iii, Majadahonda, Spain

Background: Currently mosquitoes present a significant threat to public health as vectors of numerous infectious agents; surveillance has several purposes such as gathering epidemiological data about diseases from both vector and pathogen. The genus *Flavivirus* (family *Flaviviridae*) comprises more than 70 viruses, distributed throughout the world and they are included along with other viruses, most of them zoonotic and transmitted by arthropods, i.e. West Nile virus (WNV), Japanese encephalitis virus (JEV) and Usutu virus (USUV) that represent a number of unexpected emergent flaviviruses. The goals of this study were to identify the presence of flavivirus and its potential vectors in an area that wasn't described in Spain. Not to mention the contribution of the knowledge about the mosquitoes in the studied area that could help improve the Arthropod-borne-virus surveillance programs.

Methods and Results: Mosquitoes were collected in nine representative sites and selected from a natural reserve in the middle of the Ebro Valley in Zaragoza (north-eastern Spain). During mosquito season, between April and October 2009, weekly captures were performed using BG-Sentinel traps, CDC light traps and ultraviolet traps, in which dry ice was used as a source of CO2 to attract mosquitoes. A total of 1686 mosquitoes were caught and grouped in 157 pools, representing 12 species of Culicidae. The screening was performed with a generic RT-nested-PCR to detect flavivirus genome. Two insect flaviviruses were identified, SOcFV and AEFV in the 29,2% of flavivirus positive pools.

Conclusions: Entomological studies allow generating different strategies to control vectors, transmission and diseases spreading. *Culex pipiens* was the dominant specie during the study and is the primary vector of WNV in Europe. No flavivirus pathogens were identified, besides, the two insect flavivirus could be competing in the vector with falvivirus pathogens preventing their emergence in studied area. More detailed insect flavivirus studies will help to clarify the nature and evolution of the flavivirus genus. Therefore, determining specific mosquito habitats and its annual distribution is necessary to predict the risk of disease and can be a valuable tool for the surveillance of public health threats from mosquito-borne diseases.

Board No: 92 *Ecology of Emerging Zoonoses*

MOLECULAR CHARACTERIZATION OF *BABESIA* SPP. CIRCULATING IN SLOVAKIA

B. Víchová, L. Blaňárová, V. Majláthová, B. Haklová, B. Peťko

Department of Vector Borne Diseases, Institute of Parasitology SAS, Kosice, Slovakia

In Slovakia, babesiosis was primarily a problem of the 50's of the last century. The local overgrowth of the ornate dog tick (*Dermacentor reticulatus*) populations, transmitting *Babesia divergens*, caused severe damage to livestock, particularly in the southern and southeastern Slovakia.
At the beginning of a new millennium, infections have begun to occur sporadically again, in dogs. First autochthonous report of canine babesiosis, caused by *Babesia canis*, was described in 2001 (Chandoga et al. 2001). Since then, the number of cases is growing exponentially every year. Currently, Slovakia is considered a country with the endemic occurrence of canine babesiosis.
In Europe, human infections are caused by *B. divergens*, *B. bovis* and *B. ventorum*. The first case of human babesiosis in Europe was described in 1957 in Yugoslavia (Škrabalo and Deanovic, 1957). Since then, at least 40 cases have been confirmed in Europe, including 2 infections caused by *B. microti* in patients from Switzerland and Germany (Meer-Scherrer et al., 2004, Hildenbrand et al., 2007).
Phylogenetic analyses confirmed that *B. microti* is a complex species, consisting of genetically diverse isolates that constitute three clades (Goethert and Telford 2003). Within these clades, rodent isolates are subdivided into zoonotic and nonzoonotic strains. These strains circulate in natural foci altogether or independently by different rodent species. There are assumptions that in the circulation of human pathogenic ecotypes, yellow-necked mouse (*A. flavicollis*), which is very frequent in natural foci of Slovakia, might be predominantly involved.
The main aim of our ongoing project entitled "Babesioses in Slovakia" is to determine the presence of members of the Babesiadae family in the vector ticks and hosts in Slovakia. To date, apart from *B. canis*, we have confirmed the circulation of two *B. microti* strains in ticks and rodents, with the predominant occurrence of pathogenic "Jena strain" and sporadic evidence of "Munich strain"; the presence of *B. venatorum* in ticks as well as *B. odocoilei* in deer.
The study was supported by the project of Research & Development Operational Programme funded by the ERDF (ITMS: 26220220116) (0,1); by the projects of Slovak Research and Development Agency APVV 0267-10 and VEGA 2/0113/12

Board No: 93 *Ecology of Emerging Zoonoses*

BATS AS HANTAVIRUS RESERVOIR IN AFRICA

P.T. Witkowski[1], J.F. Drexler[2], R. Kallies[2], T. Szemes[3], M. Lickova[4], E.M. Leroy[5], C. Drosten[2], D.H. Krüger[1], B. Klempa[1,4]

[1]Institute of Virology, Charité Medical School, Berlin, Germany, [2]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [3]Department of Molecular Biology, Comenius University, Bratislava, Slovakia, [4]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia, [5]Institut De Recherches Pour Le Développement, Ur178, Franceville, Gabon

Bats are recognized as one of the most important reservoir hosts for emerging human pathogens. Hantaviruses, mainly considered as rodent-borne viruses, were very recently found in African bats, too. Magboi virus was identified in slit-faced bat (Nycteris hispida) from Sierra Leone and Mouyassué virus in banana pipistrelle (Neoromicia nanus) in Côte d'Ivoire. Here we report on the detection of a new hantavirus in Noack's roundleaf bat (Hipposideros ruber).

Within the study, blood samples from 320 bats (137 H. gigas, 123 H. ruber, 60 Miniopterus inflatus) trapped near the city of Makokou, Gabon, were tested for the presence of hantavirus RNA. By using our genus-reactive nested RT-PCR screening assay targeting the large (L) genomic segment, a single positive sample, designated GB303, from H. ruber was obtained. For sequence extension, a next-generation-sequencing approach was used. Phylogenetic analyses of the L segment indicated that GB303 represents a novel distinct hantavirus, provisionally called Makokou virus (MAKV). It belongs to the newly recognised, highly divergent phylogenetic group containing hantaviruses recently identified in shrews, moles, as well as bats. Magboi virus from Sierra Leone is the most closely related virus.

In addition, a quantitative RT-PCR assay was established to determine organ tropism of the virus in the infected bat. The virus could be detected in all of the available organs (brain, gut, heart, kidney, liver, spleen) while the highest virus load was observed in spleen, kidney, and heart resembling hantavirus organ distribution in other reservoir hosts.

Altogether, identification of a third distinct hantavirus in African bats and its organ distribution in the infected bat further support the emerging concept of bats as yet overlooked hantavirus reservoir hosts.

Board No: 94 *Ecology of Emerging Zoonoses*

CFR-MEDIATED TRANSFERABLE ANTIBIOTIC RESISTANCE IN COAGULASE-NEGATIVE STAPHYLOCOCCI FROM NASAL COLONIZATION OF HUMANS WITH OCCUPATIONAL EXPOSURE TO LIVESTOCK

W. Witte[1], A. Fessler[2], S. Schwarz[3], C. Cuny[1]

[1]Wernigerode Branch, Robert Koch Institute, Wernigerode, Germany, [2]Institute of Farm Animal Genetics, Friedrich Loeffler Institute, Neustadt Mariensee, Germany, [3]Institue of Farm Animal Genetics, Friedrich Loeffler Institute, Neustadt Mariensee, Germany

Objectives: Cfr mediates resistance against antibiotics targeting the peptidyltransferase site of the bacterial ribosome (phenicols, oxazolidinones, pleuromutilins, lincosamides, streptogramin A) by dimethylation of A2503 of 23S rRNA. It was first reported from coagulase negative staphylococci (CoNS) of animal origin , later on also from livestock-associated MRSA, from nosocomial *S. epidermidis*, and also from hospital-associated MRSA in Spain . Very likely, CoNS from animals may play a role as reservoir of cfr. Here we report a study on cfr in CoNS from nasal colonization of pigs, of farmers and of veterinarians.

Methods: Nasal swabs from pigs and their farmers as well as from veterinarians were streaked on selective agar plates containing florfenicol. Colonies indicative of staphylococci were subjected to species diagnostics and to susceptibility testing by broth microdilution. Isolates growing on these selective plates were further subjected to PCR screening for cfr, followed transfer of florfenicol resistance by filter mating and detection of cfr-carrying plasmids in transconjugants.

Results: Samples from pigs in 3 among the 8 farms investigated (6 among 67 animals) contained cfr-carrying S. kloosii, S. cohnii, and S. sciuri. No cfr-carrying CoNS were detected in the swabs from 39 farmers. Only swabs from 4 among 344 veterinarians (1.2%) investigated were positive for cfr-carrying *S. epidermidis* or *S. saprophyticus*. The gene cfr was located on plasmids which were transferable to *S. aureus* 8325 by filter mating.

Conclusions: The demonstration of cfr in S. kloosii, S. cohnii, and S. sciuri from pigs but not in CoNS of pig farmers might be due to host specificity. However, members of these CoNS species have also been described from nosocomial infections in India. Although CoNS containing transferable cfr genes, such as S. epidemidis and S. saprophyticus, were rare in nasal colonization of veterinarians (1.2%), their emergence needs further attention by appropriate surveillance.

Board No: 1 *Infrastructures for Zoonoses Research*

PRIMARY DATABASE FOR MICROBIOLOGICAL DATA

T. Semmler[1], I. Semmler[2], L.H. Wieler[1]

[1]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [2]TMF – Technology, Methods, and Infrastructure for Networked Medical Research, Zoonoses and Infection Research Working Group, Berlin, Germany

Here we introduce an open source client server application which allows the standardized and structured storage of data generated in microbiological research.
On the basis of a PostgreSQL database the application allows the access via a web browser to the strain centric data. Administrative data to the strains as well as epidemiological and molecular data can be stored. Any kind of binary data plus sequence data up to whole genomes, for which a BLAST search was included can be stored without limitations.
The database scheme that was developed with various German research networks for zoonoses together with several universitary and federal research institutions. This can lead to standardized data collections and exchanges for future collaborations

Board No: 2 *Infrastructures for Zoonoses Research*

STRIVING TOWARD ONE HEALTH: EVOLUTION OF INTERNATIONAL COLLABORATION NETWORKS ON NIPAH VIRUS RESEARCH FROM 1999 TO 2011

S.F. Valeix

Institute of Development Studies, University of Sussex, Brighton, United Kingdom

The One Health concept is supported by international institutions in response to emerging zoonotic viruses as major threats for global public health. One Health proposes to enhance scientific cooperation between various disciplines and on a worldwide scale. This paper examines the evolution of research networks on the emerging Nipah virus, from 1999 to 2011. It combines social network analysis on bibliometric data and interviews with co-authors, actors of collaboration networks. The main conclusion of this study is that, despite an important role of knowledge brokerage played by low and middle income countries right after Nipah's emergence, these countries lost their central position in the networks over time. This conferred to high income countries the power of managing international flows of knowledge about the disease and thus a consequent responsibility in linking communities and fields in the regard to One Health principles. Moreover, the involvement of the private sector and interdisciplinary research directly oriented at the interface between human, animal and environmental health remain poor. The identification of changing patterns of scientific cooperation on Nipah virus over time could inform policies that aim to develop efficient networks to meet the challenge of emerging zoonoses.

Board No: 3 *Pathogen-Cell Interaction and Immunity*

INVESTIGATIONS ON THE ANTIGENICITY OF MYCOBACTERIAL LIPOPEPTIDES

E. Kaufmann[1], C. Spohr[1], M. Gilleron[2], R. Bauerfeind[3], E. Balks[1], K. Cussler[4], M. Bastian[1]

[1]Division of Veterinary Medicine, Paul Ehrlich Institut, Langen, Germany , [2]Institute of Pharmacology and Structural Biology, Centre Nationale De La Recherche Scientifique, Toulouse, France , [3]Institute of Veterinary Hygiene and Infectious Diseases, Justus Liebig University, Giessen, Germany , [4]Safety of Veterinary Products, Paul Ehrlich Institut, Langen, Germany

Tuberculosis is still one of the most important and life-threatening diseases worldwide. It is caused by mycobacteria of the tuberculosis complex, e.g. the zoonotic agents *M. tuberculosis* and *M. bovis*. Improved diagnostic tools and reliable, effective prevention strategies are urgently required. Two recent publications (Bastian et al., JI, 2008; Seshadri et al., JI, 2013) describe a highly immunogenic class of MHC-II-restricted, mycobacterial lipopeptides.

The aim of this project is to further characterize the immunogenicity of this so far uncharacterized class of antigens *in vitro* and *ex vivo* using guinea pigs as small animal model. Guinea pigs are sensitive to mycobacterial infections and develop a similar disease pattern like humans or cattle.

To test for the immunogenicity of lipopeptides *ex vivo* we use PBMCs of BCG-sensitized guinea pigs and perform T-cell proliferation assays using mycobacterial Chloroform-Methanol-Extracts (CME) and a lipopeptide enriched subfraction. For comparison we use Tuberculin, the most widely used diagnostic TB antigen. CME and the subfraction induce a robust T-cell proliferation which is comparable to the tuberculin response. Protease treatment and delipidation of CME or of tuberculin significantly reduces antigen-specific T-cell proliferation. This indicates that the respective antigens contain a peptide- and a lipid-moiety, which are both important for their stimulatory potential. In restimulation assays we observe that tuberculin-specific T-cells respond comparably to tuberculin and CME and vice versa. This indicates that the respective highly immunogenic antigens are present in both preparations although they differ substantially in their composition.

From our observations we conclude that mycobacterial lipopeptides are highly immunogenic and induce a strong and antigen-specific T-cell response. Ongoing efforts aim to define these new lipopeptide antigens and to determine whether the lipopeptides are specific for mycobacteria of the tuberculosis complex.

Board No: 4 *Pathogen-Cell Interaction and Immunity*

LIPOTEICHOIC ACID OF *STAPHYLOCOCCUS AUREUS* AS MAIN CONTRIBUTOR TO THE ENHANCEMENT OF INFLUENZA A VIRUS INDUCED MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING

C. Klemm[1], K. Warnking[1], B. Löffler[2], G. Peters[2], S. Ludwig[1], C. Ehrhardt[1]

[1]Institute of Molecular Virology, Westfaelische Wilhelms University Muenster, Muenster, Germany, [2]Institute of Medical Microbiology, University Hospital of Muenster, Muenster, Germany

Bacterial co-infections are a major complication of influenza A virus (IAV) infections leading to severe illness and fatal outcomes. Recent findings suggest that beside the pathogen load, a dysregulated immune response of the host also contributes to increased morbidity and mortality. Toll-like receptors (TLRs) play an important role in the innate immune response by pathogen sensing and activating signaling cascades leading to the induction of pro-inflammatory cytokines and chemokines. Although several *in vivo* studies demonstrate elevated levels of cytokines and chemokines upon IAV and bacterial co-infections resulting in a massive influx of immune cells into the lung and severe tissue damage, the underlying molecular signaling mechanisms still remain to be elucidated. However, this knowledge is crucial for development of new therapeutic approaches.

In the present study we focused on cellular signaling mechanisms in a human lung epithelial cell line (A549) resulting in a dysregulated innate immune response upon co-infection with IAV and *Staphylococcus aureus* (*S. aureus*). We established an *in vitro* co-infection protocol in A549 cells including a serial pathogen incubation combined with an antibiotic wash.

Upon co-infection with IAV and *S. aureus* we observed elevated levels of cytokines and chemokines as described in *in vivo* models. Analyses of cellular signaling mechanisms regulating these innate immune response genes revealed significantly increased activation of the mitogen-activated protein kinases (MAPKs) JNK and p38 in presence of both pathogens compared to IAV-infected cells. Similar results were obtained, when IAV infection was replaced with viral RNA or *S. aureus* infection was restored by lipoteichoic acid (LTA) stimulation, but not with other bacterial components.

Our data indicate a correlation of hyper-activation of MAPKs and overexpression of pro-inflammatory cytokines and chemokines to the activation of TLRs. We will provide deeper insights in the regulation of pathogenicity during IAV and *S. aureus* co-infections on a molecular level, which contributes to the lethal synergism of these pathogens.

Board No: 5 *Pathogen-Cell Interaction and Immunity*

CHARACTERIZING THE INTERACTION OF *CHLAMYDIA PSITTACI* WITH THE GOLGI APPARATUS – A COMPARATIVE ANALYSIS OF A HUMAN AND ZOONOTIC PATHOGEN

S. Koch, L. Aeberhard, S. Banhart, A. Herrmann, D. Heuer

Junior Group 5 Sexually Transmitted Bacterial Pathogens, Robert Koch Institute, Berlin, Germany

The zoonotic pathogen *Chlamydia* (*C.*) *psittaci* is unique among the species of *Chlamyidacea* regarding its remarkably wide host range, the severe course of clinical disease caused in some humans contrasted to the high proportion of latent infections not leading to overt disease. Today the molecular mechanisms leading to associated disease are not well understood.

In this study, we analyzed the interaction of *C. psittaci* with the cellular Golgi apparatus (GA) in comparison to the human pathogen, *C. trachomatis*. *C. trachomatis* fragments the Golgi apparatus and Golgi mini stacks are then recruited to the bacterial inclusion. This Golgi fragmentation is a hallmark of *C. trachomatis* infections at least in human cells and it supports bacterial sphingolipid acquisition and progeny formation. *C. psittaci* infections also lead to Golgi fragmentation. In contrast to *C. trachomatis*, *C. psittaci* induces fragmentation of the GA into smaller Golgi elements that show increased spreading. This spreading phenotype is seen for several *C. psittaci* strains isolated from different mammals including cattle, swine and human. Moreover, distinct Golgi proteins, mediating transport in the GA or between GA and plasma membrane are not associated with the *C. psittaci* inclusion indicating that *C. psittaci* and *C. trachomatis* interact differently with the Golgi apparatus. Further characterization of these specific features of *C. psittaci* infections will improve our understanding of the molecular pathogenesis of this zoonotic agent and may open new routes for the development of novel therapeutic concepts.

Board No: 6 *Pathogen-Cell Interaction and Immunity*

INHIBITION OF ALPHAVIRUSES BY THE HUMAN ZINC FINGER ANTIVIRAL PROTEIN

S. Gläsker, M. Töller, B.M. Kümmerer

Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

The zinc finger antiviral protein (ZAP) is characterized by four CCCH-type zinc finger motifs in the N-terminal part of ZAP, which are thought to be the major functional domain with regard to antiviral activity. ZAP is described to inhibit the viral translation and mediates degradation of viral RNAs by directly binding to the viral mRNA and recruiting the RNA exosome. The human (h) ZAP gene is spliced to yield a short (hZAP-S) and a long (hZAP-L) isoform. While both isoforms contain the N-terminal zinc finger motifs, hZAP-L contains an additional poly(ADP-ribose) polymerase (PARP)-like domain in its C-terminus.

In contrast to bona fide PARPs, which are characterized by the triad motif HYE, the PARP-like domain of hZAP-L exhibits the triad motif YYV instead. Due to this alteration, hZAP-L is predicted to be catalytically inactive thereby questioning the importance of the PARP-like domain for its antiviral activity. Using stably transfected human 293 cells based on the Flp-In T-REx system and Sindbis virus as prototype member of alphaviruses we confirmed that hZAP-L is a more potent inhibitor of alphaviruses than hZAP-S. Furthermore, specific siRNA-mediated knockdown of hZAP-L but not hZAP-S demonstrated a role of endogenous hZAP-L in restriction of alphavirus replication. Whilst single amino-acid substitutions in the triad motif of hZAP-L's PARP-like domain reduced the antiviral activity, exchange of all three triad motif residues to alanine or to the amino acids of active PARPs virtually abolished the antiviral effect. Contrary to previous assumptions these results indicate an essential function of the PARP-like domain in hZAP-L´s antiviral activity.

Additional studies demonstrated that hZAP inhibits different alphaviruses to different levels. Using a reverse genetics approach, current analyses investigate whether the observed difference is linked to different sensitive ZAP responsive elements in the alphavirus genomes or whether the alphaviral macrodomain encoded by the nonstructural protein nsP3 might function as possible antagonist of hZAP. These analyses should shed light on the interplay between alphaviruses and the antiviral protein hZAP.

Board No: 7 *Pathogen-Cell Interaction and Immunity*

A SEMI-IMMUNOCOMPETENT MOUSE MODEL TO STUDY EBOLAVIRUS IMMUNITY

A. Lüdtke[1,2], L. Oestereich[2], S. Günther[2], C. Muñoz-Fontela[1,2]

[1]Emerging Viruses, Heinrich Pette Institute, Hamburg, Germany, [2]Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Ebolaviruses (EBOV) are zoonotic RNA viruses from the *Filoviridae* family that cause severe disease in humans and non-human primates. Despite their importance as human pathogens very little is known about their host immune responses, especially *in vivo*. This is primarily due to the lack of immunocompetent small animal models of infection, and the restriction of EBOV research to biosafety level 4 containment.
Since immunocompetent mice are resistant to EBOV, until now, most *in vivo* studies have used mice deficient in the type I interferon signaling pathway. However, these models are not ideal to study immune responses to viruses due to their lack of the main antiviral defense mechanisms. Based on this need, our aim was to create a chimeric semi-immunocompetent mouse model by transplantation of bone marrow cells from wild-type mice into lethally irradiated IFNAR-/- recipient mice. We generated animals with a stromal compartment susceptible to viral replication but with a fully immunocompetent hematopoietic system. Our data indicated that the chimeric mice supported productive EBOV infection and showed enhanced survival compared to IFNAR-/- mice. This mouse model allowed us to study the kinetics of immune responses and mechanisms that regulate the initiation of adaptive immunity to EBOV *in vivo*.

Board No: 8 *Pathogen-Cell Interaction and Immunity*

AMINO ACID 11 IS ESSENTIAL FOR THE ANTI-APOPTOTIC ACTIVITY OF THE *COXIELLA BURNETII* EFFECTOR ANKG

R. Eckart, W. Schäfer, A. Lührmann

Universitätsklinikum Erlangen, Mikrobiologisches Institut, Erlangen, Germany

The obligate intracellular bacterium *Coxiella burnetii* is the causative agent of the zoonotic disease Q-fever. Q-fever is often a mild flu-like illness, but can develop into an atypical pneumonia or hepatitis. Furthermore, the infection can lead to chronic infection which is typically characterized by bacterial endocarditis and is potentially fatal. *C. burnetii* pathogenesis depends on a functional type IV secretion system (T4SS), used to translocate bacterial proteins into the host cell in order to manipulate host cell pathways. To date over 100 effector proteins have been identified, however their functions mainly remains elusive. We have demonstrated that the T4SS effector AnkG inhibits host cell apoptosis and it is believed that this activity is essential for the establishment of a persistent infection. However, the mode of action of AnkG is still not fully understood.
Here, we compared the activity of AnkG encoded by different *C. burnetii* strains. The differences between the AnkGs from *C. burnetii* Nine Mile, Dugway and G isolate are amino acid exchanges at position 11 and 72. Although there are only two amino acids exchanged, we observed a difference in anti-apoptotic activity. Thus, *C. burnetii* Dugway displayed a significantly increased anti-apoptotic activity compared to *C. burnetii* Nine Mile. *C. burnetii* Dugway contains an Isoleucine at aa 11 whereas *C. burnetii* Nine Mile contains an Leucine at this position. To investigate the role of amino acid 11 in inhibition of apoptosis, we constructed different AnkG mutants exchanging Isoleucine to either Glutamic Acid (AnkGI11E), to Threonine (AnkGI11T) or to Valine (AnkGI11V). Our results demonstrate that the expression of these three AnkG mutants had no inhibitory effect on staurosporine-induced apoptosis, while the expression of GFP-AnkG and -AnkGI11L inhibited staurosporine-induced apoptosis. Thus, the amino acids Isoleucine or Leucine, but not amino acids Glutamic acid, Threonine or Valine at position 11 of AnkG is required for anti-apoptotic activity. This indicates that the region around amino acid 11 is the active anti-apoptotic region of AnkG, which fits with previous observations that the first 69 amino acid of AnkG are necessary and sufficient for anti-apoptotic activity.

Board No: 9 *Pathogen-Cell Interaction and Immunity*

IN VITRO DETECTION OF CELL DEATH IN HUMAN CELL LINES INFECTED BY TICK-BORNE ENCEPHALITIS VIRUS

A. Mayer[1], K. Brauer[1], S. Frey[1], G. Dobler[1], R. Kühn[2], S. Eßbauer[1]

[1]Laboratory of Microbiology, Bundeswehr Institute of Microbiology, Munich, Germany, [2]Laboratory of Microbiology, Technical University of Munich, Munich, Germany

Tick-borne encephalitis (TBE) is one of the most important and frequent inflammatory zoonotic diseases of the central nervous system. In Europe and Asia, the tick-borne encephalitis virus (TBEV) causes more than 10.000 infections per year with endemic foci from Japan to France. TBEV might cause severe meningo-encephalitis with a fatality rate right up to 15 % - depending on strain or subtype. As the neuropathogenic mechanism is not known in detail, the aim of this project was to analyse cell death gene expression of TBEV-infected cells *in vitro*.

To study gene expression after TBEV infection *in vitro*, cell death was investigated in human cell lines like DBTRG (glioblastoma) and SIMA (neuroblastoma) and the African green monkey cell line VeroB4. Either these cell lines were infected with TBEV isolates or apoptosis, necrosis and autophagy were induced in cells using reagents such as staurosporine, ionomycin and rapamycin.

The efficiency of cell death induction was confirmed by visualization with light microscope, DAPI-staining, TUNEL-assay, cytotoxicity-assay, autophagosome visualization and measurement of impedance. Gene expression was investigated using both established semi quantitative real time PCRs as control assays and human cell death RT2 ProfilerTM PCR Arrays. These arrays permit to detect different cell death specific genes, activated by TBEV infection, to generate gene expression profiles of infected cells in comparison to cell death induced cells. Thus it might be possible to characterize genetic modifications caused by TBEV infection and to define activated cell death pathways of infected cells.

Board No: 10 *Pathogen-Cell Interaction and Immunity*

SUSCEPTIBILITY OF PULMONARY CELLS TO OLD WORLD HANTAVIRUSES

A. Müller, A. Baumann, B. Burkard, E. Asberger, M. Zeier, E. Krautkrämer

Department of Nephrology, Univeristy of Heidelberg, Heidelberg, Germany

Hantaviruses are emerging zoonotic pathogens that are transmitted to humans by inhalation and cause disease referred to as hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Recently, reports accumulate that Old World and New World hantavirus-infected patients may display both renal and pulmonary symptoms, shifting the classical paradigm of HFRS and HCPS towards the more general term "hantavirus disease". In particular, infection with the Old World hantavirus Puumala (PUUV) can lead to pulmonary involvement, with a clinical picture resembling that of HCPS-causing hantaviruses.
Given the route of transmission and pulmonary involvement of Old World hantaviruses, pulmonary cells are likely to represent target cells for hantaviruses during the early stage of infection. Therefore, we sought to investigate epithelial and endothelial pulmonary cell types for their susceptibility to hantavirus infection. We found that PUUV and HTNV establish infection in human primary small airway epithelial cells (HSAEpC) and pulmonary microvascular endothelial cells (HPMEC) cells *in vitro*, indicating that pulmonary cells are susceptible to Old World hantaviruses. Moreover, scratch assays with the bronchial epithelial cell line Calu-3 revealed that infection was dependent on both cell density and monolayer integrity. Using flow cytometry and Western blot analysis, pulmonary cells were examined for expression of hantaviral receptors. All cell types expressed the hantaviral attachment factor CD55. Interestingly, integrin αvβ3, which is utilized by pathogenic hantaviruses as an entry receptor, was detectable on endothelial HPMEC but not on the epithelial HSAEpC and Calu-3 cells.
Together, the data suggest that respiratory cells are involved in the early stage of hantavirus disease, but that the entry mechanism may differ between epithelial and endothelial pulmonary cell types. Further studies are necessary to analyze the role of receptor abundance and accessibility for the infection of pulmonary cells and will help to understand the pathogenic mechanisms contributing to pulmonary involvement of Old World hantaviruses.

Board No: 11 *Pathogen-Cell Interaction and Immunity*

CONSERVED ANTI-INTERFERON ACTIVITY OF PROTEIN 4a HOMOLOGUES FROM MERS-CoV AND RELATED ANIMAL BETACORONAVIRUSES

J. Papies[1], D. Ritz[1], D. Niemeyer[1], T. Zillinger[2], E. Klein[1], A. Sieberg[1], D. Muth[1], V.M. Corman[1], G. Horvath[3], F. Zielecki[4], T. Suliman[1], W. Barchet[2], J.F. Drexler[1], F. Weber[4], C. Drosten[1], M.A. Müller[1]

[1]Institute of Virology, University of Bonn Medical Center, Bonn, Germany, [2]Institute of Clinical Pharmacology, University of Bonn Medical Center, Bonn, Germany, [3]Institute of Innate Immunity, University of Bonn Medical Center, Bonn, Germany, [4]Institute for Virology, Philipps University Marburg, Marburg, Germany

The highly pathogenic human betacoronavirus Middle East respiratory syndrome (MERS)-CoV was shown to inhibit the cellular interferon (IFN) response. We previously demonstrated that MERS-CoV accessory protein p4a is a potent IFN antagonist. A zoonotic origin of MERS-CoV is suspected. Here we characterized p4a homologues from two related betacoronaviruses detected in *Nycteris* bats and hedgehogs and assessed putative anti-IFN functions.

P4a homologues showed only 40% amino acid identity with MERS-CoV but shared conserved dsRNA-binding motifs. In IFN promoter reporter assays all three p4a inhibited the activation of the IFN response and blocked the nuclear translocation of IFN regulatory factor 3 in human cells.

In summary, p4a from distantly related CoV were proficient IFN antagonists in human cells without adaptation.

Board No: 12 *Pathogen-Cell Interaction and Immunity*

EFFECT OF NEOSTIGMINE ON TULAREMIA PROGRESSION IN BALB/C MICE

M. Pohanka[1], O. Pavlis[2]

[1]Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic, [2]Centre of Bioclogical Defence, Army of Czech Republic, Techonin, Czech Republic

Neostigmine is a pseudo-irreversible inhibitor of enzyme acetylcholinesterase (AChE). We hypothesize link between neostigmine and cholinergic anti-inflammatory pathway via better availability of blood acetylcholine after AChE inhibition and consequent activation of nicotinic acetylcholine receptors. Owing to the expected mechanism of action, we expect significant impact of neostigmine on immunity. In the reported experiment, we used BALB/c mouse model and experimental infection with Francisella tularensis, a causative agent of tularemia. Interferon γ, interleukin 6 and mortality test were done for neostigmine doses 8.00, 40.0, and 200 μg/kg. We proved significant decrease of the both cytokines in course of neostigmine in a dose dependent manner. Neostigmine aggravated mortality caused by tularemia as well. Owing to the reported results, application of AChE inhibitors in patients suspected from tularemia or similar diseases can be considered as a life endangering therapy.

A long-term organization development plan 1011 (Faculty of Military Health Sciences, University of Defence, Czech Republic) is acknowledged for financial support.

Board No: 13 *Pathogen-Cell Interaction and Immunity*

ACTIVATED BOVINE BLOOD LEUKOCYTE KINETICS PARALLEL THE CLINICAL COURSE AFTER INTRABRONCHIAL INOCULATION WITH *CHLAMYDIA PSITTACI*

A. Prohl, M. Lohr, C. Ostermann, K. Sachse, C. Menge, P. Reinhold, A. Berndt

Institute of Molecular Pathogenesis, Friedrich Loeffler Institut, Federal Institute for Animal Health, Jena, Germany

Infection of cattle with chlamydiae, such as *Chlamydia (C.) psittaci*, is very widespread and has a quantifiable impact on livestock productivity, with acute respiratory disease and chronic recurrent infections being typical manifestations. The implication of the systemic cellular immune response in the initial phases of disease is unknown. It was the aim of this study to characterize the phenotype and activation state of peripheral blood lymphocytes, monocytes and granulocytes in a recently established model of bovine acute respiratory *C. psittaci* infection.

Seven calves were inoculated intrabronchially with 10e+08 inclusion forming units of *C. psittaci* at 6-8 weeks of age. Blood was sampled 7 days, 4 days and 1 hour before inoculation and 1, 2, 3, 5, 7 and 10 days after inoculation (dpi) for flow cytometry analysis of leukocyte surface markers. Animals were clinically examined daily and necropsied 14 dpi. At necropsy, pieces of lung and mediastinal lymphnode were sampled for quantitative real-time PCR. Successful inoculation was confirmed by the presence of clinical signs of respiratory disease in all animals lasting from 2 dpi to 7 dpi and by the presence of chlamydial DNA in tissue 14 dpi.

As compared to pre-inoculation values, absolute numbers of CD4+ and CD8α high T cells significantly dropped at 2 dpi, particularly affecting CD8α high lymphocytes and resulting in an increase of the CD4+/CD8α high ratio. Changes were accompanied by significantly increased CD25 expression on CD8α dim/CD62L+ lymphocytes and on a CD62L+ subset, which was CD4-CD8α-. Monocytes significantly increased expression of CD11b, with CD14+ monocytes exhibiting a marked increase of MHCII and CD14 expression peaking at 2 and 3 dpi. The significant increase of CD62L, CD11b and CD25 expression on granulocytes followed the same kinetics. Expression levels of activation markers and adhesion molecules on the surface of all leukocyte subsets returned to baseline levels at 10 dpi the latest.

C. psittaci-infected calves undergoing acute respiratory disease exhibit a rapid and systemic activation of the immune system leading to an effective immune control of the infectious agent.

Board No: 14 *Pathogen-Cell Interaction and Immunity*

MASS-SPECTROMETRY BASED PROFILING OF PKR-INTERACTION PARTNERS IN THE COURSE OF INFLUENZA A VIRUS INFECTION

S. Sänger, R. Davina Nunez, T. Wolff

Division of Influenza and Other Respiratory Viruses, Robert Koch Institute, Berlin, Germany

PKR is an interferon induced, double-stranded RNA-activated protein kinase, which plays a significant role in innate immunity. The activation of PKR by the recognition of viral nucleic acids, bacterial stimuli or stress leads to dimerization, autophosphorylation and phosphorylation of numerous downstream factors. Downstream effects of PKR activation include inhibition of translation, initiation of apoptosis and the induction of transcription factors that lead to production of type I interferon. Due to its key role in antiviral immunity many viruses have evolved mechanisms to avoid PKR activation. We and others have previously described the influenza virus non-structural protein 1 (NS1) as a protein antagonist of PKR. We are interested in understanding the precise mechanism of PKR activation in the context of influenza virus infection and the role of cellular and viral factors in regulating PKR activation. For this purpose we used Stable Isotope Labeling by Amino acids in Cell culture (SILAC) followed by LC-MS/MS analysis to identify immunoprecipitable interaction partners of PKR before and during influenza virus infection. Labeled cells were infected with wildtype or delNS1 virus to compare the effect of the viral antagonist on the PKR interactome. In four replicates we were able to detect more than 180 cellular PKR binding partners including several proteins previously described as PKR interactors such as eIF2α, HSP90 and PP2A. Among the detected proteins 54 interactors were only found in wildtype infected cells whereas 29 were characteristic for delNS1 virus infection. Bioinformatic analysis indicates that the majority of these particular proteins are involved in cellular pathways as RNA processing, stress response and apoptosis. We are in the process of validating a subset of the detected proteins by co-immunoprecipitation and immunofluorescence experiments. It is expected that bioinformatic and functional analyses of novel PKR interaction partners will further our understanding of cellular antiviral mechanisms and their modulation by influenza A virus.

Board No: 15 *Pathogen-Cell Interaction and Immunity*

EPITHELIAL RECEPTOR NECTIN4 INTERACTIONS ARE REQUIRED FOR EFFICIENT MORBILLIVIRUS TRANSMISSION

B. Sawatsky, V. von Messling

Division of Veterinary Medicine, Paul Ehrlich Institut, Langen, Germany

The highly contagious morbilliviruses measles virus (MeV) and canine distemper virus (CDV) still cause a significant worldwide disease burden in humans and animals despite the availability of effective vaccines for both viruses. The determinants of their efficient transmission from infected to naive hosts and the roles of the different cellular receptors in this process are not fully understood. Towards this, we housed naive ferrets for varying times with animals that were infected with either wild-type virus or mutant viruses that no longer recognize the immune cell receptor signaling lymphocyte activation molecule (SLAM) or the epithelial receptor nectin4. From six days after infection with wild-type virus, contact animals became reliably infected, but transmission at earlier times was less efficient. Consequently, contact animals succumbed to disease approximately one week after the infected animals. We next assessed the importance of cellular receptor usage in transmission by infecting animals with SLAM- or nectin4-blind viruses, respectively, and co-housing them with naive animals starting three days after infection. Nectin4-blind virus was only transmitted between one infected/contact pair, while none of the other contact animals became infected. Interestingly, the infected animal in the pair that transmitted virus had a higher peak virus titer, and remained viremic for one week longer than the other infected animals. The contact animal became viremic between days 17 and 21 after initial infection and did not develop clinical signs. In the group infected with the SLAM-blind virus, only one animal had a transient viremia on day 7 post-infection, but the virus was not transmitted to any of the naive contacts. Our results indicate that efficient CDV transmission begins approximately one week after infection, briefly preceding the development of clinical signs, which reproduces the timing of transmission known for MeV. Furthermore, transmission requires interaction with both cellular receptors, thereby confirming the role of nectin4 as an exit receptor.

Board No: 16 *Pathogen-Cell Interaction and Immunity*

ROLE OF NS1 PHOSPHORYLATION FOR INFLUENZA A VIRUS REPLICATION IN HUMAN LUNG EPITHELIAL CELLS

T. Schräder, C. Nordhoff, D. Anhlan, V. Wixler, S. Ludwig

Institute of Molecular Virology, Centre for Molecular Biology of Inflammation, Westfaelische Wilhelms Universitaet, Muenster, Germany

Pandemics as well as seasonal outbreaks are a hallmark of influenza A virus (IAV) infections, making it an important health threat. IAV has evolved several strategies to counteract host immune responses, such as the production of antiviral acting interferons. The non-structural protein 1 (NS1) has been identified as a major interferon antagonist of the virus. The protein is only expressed in infected cells but not incorporated into progeny virions. IAV NS1 is a small multifunctional protein that is implicated in several crucial processes in the viral life cycle. It dampens host immune responses and enhances viral replication by interfering with host cell metabolism.
Many cellular processes are regulated by phosphorylation and dephosphorylation. In a phosphoproteomic analysis of A549 cells infected with IAV, we found several viral proteins to be differentially phosphorylated during the viral life cycle. With regard to NS1 we were able to identify several phosphorylated amino acid residues, some of which were previously unknown. This prompted us to investigate the role of NS1 phosphorylation for viral replication in detail. Using reverse genetics we generated a set of influenza A/PR/8/34 (H1N1) virus mutants. These mutants encoded NS1 with phosphoacceptor sites mutated into amino acids that either mimic a constitutive phosphorylation (negatively charged residue: e.g. D or E) or that cannot be phosphorylated (e.g. A, G or L).
We analysed the mutants by investigation of viral mRNA and protein expression level as well as progeny virus titers. First data indicates that NS1 threonine (T) residue 215 (T215) is crucial for virus growth, at least in cell culture using A549 cells. Mutations resulted in attenuated virus growth. Mimicked constitutive phosphorylation (T215D mutant) strongly reduced viral titers at later time points of infection. In addition, the T215L mutation in NS1 also affected viral titers. Based on this we will further investigate the molecular mechanism leading to reduced virus replication of the NS1 T215L and T215D mutant. (These data were also in part presented at the International Influenza Meeting 2014, Münster, Germany Sep 21-23, 2014)

Board No: 17 *Pathogen-Cell Interaction and Immunity*

COMPARATIVE CHARACTERIZATION OF COWPOX VIRUS ISOLATES WITH DIFFERENT PATHOGENICITY

L. Schuenadel, A. Despang, A. Nitsche

Centre for Biological Threats and Special Pathogens 1, Robert Koch Institute, Berlin, Germany

Cowpox viruses (CPXV) belong to the genus *Orthopoxvirus* (OPV) within the *Poxviridae* family. Similar to other OPV such as monkeypox virus or vaccinia virus, CPXV have the potential to infect humans with varying severity of disease. Therefore, it is vital to fully explore the viral pathogenesis of CPXV infections, including the underlying molecular mechanisms. Since CPXV have the most complete set of immunomodulatory proteins, they are an ideal model to study virus–host interactions.
Recently, *in vivo* experiments performed with Wistar rats revealed an increased virulence of the wild-type isolate CPXV Hei compared to that of the reference strain Brighton Red (BR). Therefore, the aim of this work was to identify the molecular differences in the varying pathogenicity of CPXV BR and CPXV Hei by comparative virologic characterization of the two viruses.
Comparing the infection with the two CPXV isolates in HeLa cells, it could be shown that the viral replication was similar for the two isolates in terms of virus reproduction and early-, intermediate- and late-stage gene expression. However, after infection with CPXV Hei a slight delay in genome replication could be observed compared to the reference strain. Additionally, the dispatch of infected cells proceeded more rapidly and formation of syncytia was enhanced after infection with CPXV Hei, which might be attributed to an increased expression of the viral protein L1R.
The next step for further identification of molecular differences during the *in vitro* infection with the two viruses is the comparison of the viral and cellular transcriptomes at different times post infection. Further examination of the identified differentially expressed cellular genes and their associated pathways will provide additional insights into the molecular mechanisms of CPXV pathogenesis.

Board No: 18 *Pathogen-Cell Interaction and Immunity*

IN VITRO-COMPARISON OF THE INTERACTION OF THE GERMAN *E. COLI* O104:H4 OUTBREAK STRAIN WITH BOVINE AND HUMAN INTESTINAL EPITHELIAL CELLS

S.A. Stalb, S. Barth, L. Geue, C. Menge

Friedrich Loeffler Institut/ Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis, Jena, Germany

In 2011, Germany was struck by the largest outbreak of hemolytic uremic syndrome. The highly virulent *E. coli* outbreak strain LB226692 possesses a blended virulence profile combining genetic patterns of human adapted enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC), a subpopulation of Shiga toxin (Stx)-producing *E. coli* (STEC). STEC are basically adapted to the bovine host but are capable of causing severe diseases in humans. Bacterial adaption to different hosts depends on specific molecular interactions which are insufficiently unravelled regarding LB226692. Aim of this study was to compare strain-specific adherence properties of different *E. coli* strains to intestinal epithelial cells of human (CaCo-2, Int407) and bovine (FKD-R 971) origin.

Cell lines were incubated for 6 hours with equal numbers of the outbreak strain, *E. coli* strains representing different pathovars or non-pathogenic *E. coli* strains. Adhesion assays were conducted to analyze pattern and rate of adhesion (Giemsa) and fluorescent actin staining (FAS) to visualize bacteria-associated actin accumulation. Level of invasion was determined by gentamicin protection assays (cfu). Amounts of Stx released upon host cell contact were quantified by ELISA.

In general, adherence properties to epithelial cells varied qualitatively (type, pattern) and quantitatively (extend) between strains and only low numbers of *E. coli* were found intracellularly. Bovine FKD-R 971 and human Int407 cells were strongly colonized by all *E. coli* strains, while only low bacterial numbers were detected on CaCo-2 cells. Quantitatively, the outbreak strain associated with intermediate numbers compared to classical EHEC strains (EDL933, 86-24WT). LB226692 adhered to any of the host-specific cell lines in characteristic stacked-brick adhesion pattern without inducing actin accumulation. For classical EHEC strains, the relative amount of Stx correlated well with the level of adhesion. Compared to classical EHEC, strain LB226692 released lower amounts of Stx to the supernatant. However LB226692 released notably higher levels of Stx when attached to bovine FKD-R 971 cells as compared to all human cells. The results point to a milieu-dependent control of virulence gene expression by LB226692 which is independent of bacterial attachment to epithelial cells.

Board No: 19 *Pathogen-Cell Interaction and Immunity*

SPECIES-SPECIFIC DIFFERENCES IN INNATE IMMUNE RESPONSES TO FLAGELLA - POSSIBLE ROLE IN HOST-ADAPTATION

K. Straßl, S. Maurischat, P. Schwerk, K. Tedin

Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany

Salmonella serovars show a large variation in their host range and degree of host adaptation, some persisting in certain animal hosts, but also capable of infecting humans. Our project has been concerned with determining the role of pathogen-associated molecular patterns (PAMPs) such as flagellin or lipopolysaccharides in the host-specificity of *Salmonella enterica* serovars.
Reporter cell lines of porcine, avian and human origin harbouring chromosomally-integrated NF-kB-dependent luciferase reporter fusions were used to systemically compare infections with *Salmonella* mutants possessing deletions in PAMP-relevant genes and the host cell innate immune response to the infection.
Species-specific differences in host innate immune responses to infection were observed. Interestingly, the loss of flagellin reduced NF-kB activation in human macrophages as expected, but strongly increased NF-kB activation in chicken macrophages, regardless of the type of mutation affecting the flagellar biosynthesis.
As the serovars *Salmonella gallinarum* and *Salmonella pullorum* are naturally non-motile, these results suggest that the loss of flagellin may play a role in the host adaptation to the avian host. Future work will focus on these aspects to better understand the underlying principles in host specificity of *Salmonella*.

Board No: 20 *Pathogen-Cell Interaction and Immunity*

ESTABLISHMENT OF CHICKEN ENTEROCYTE CELL LINES AS PRIMARY INFECTION TARGET FOR SALMONELLA AND CAMPYLOBACTER INFECTION STUDIES

Z.K. Szatanik, D. Schulte, A. Bruchmann, L. Gonzaléz, A. Dreusch, W. Rudy

Research and Development, Micromol Gmb H, Karlsruhe, Germany

The two most important zoonotic pathogens causing gastroenteritis are Campylobacter- and Salmonella spec. provoking together about 24% of food-borne diseases in the US. [1] The primary infection target is represented by the avian (chicken) enterocyte. It seems reasonable that by reduction of the pathogen load in chicken, the main source of infection, the risk for consumers to develop illness could be reduced [2]. To come away from time and money consuming field studies we aimed to implement an *in vitro* testing platform enabling the interference in the zoonotic infection process. Hereby cells were isolated from the intestine of embryonic chicken derived from pathogen free eggs. Cells were immortalized with a combination of genetic transformation and growth factor dependent propagation. Upon sophisticated cloning steps 224 immortal clones of the embryonic chicken could be established representing at least 6 different morphotypes. In further steps these cell lines have than been characterized for their chicken origin and epithelial properties.
An assay for infections studies with the zoonotic pathogens Salmonella and Campylobacter based on this newly developed cell lines was established. This system might represent a powerful tool identify infection inhibitors *in vitro* and therefore could be used to reduce animal trials in poultry feed additive research. Furthermore, this assay might be extended for other pathogens.

[1] Tauxe, R. 2002. Emerging food-borne pathogens. Int. J. Food Microbiol. 78:31-41.
[2] Meerburg B G, Kijlstra A. 2007. Review Role of rodents in transmission of Salmonella and Campylobacter. J Sci Food Agric
87:2774–2781

Board No: 21 *Pathogen-Cell Interaction and Immunity*

***STAPHYLOCOCCUS AUREUS* INHIBITS INFLUENZA A VIRUS-INDUCED APOPTOSIS**

A. van Krüchten[1], C. Klemm[1], B. Löffler[2], G. Peters[2], S. Ludwig[1], C. Ehrhardt[1]

[1]Institute of Molecular Virology, Center for Molecular Biology of Inflammation, Westfaelische Wilhelms University of Muenster, Muenster, Germany, [2]Institute of Medical Microbiology, University Hospital of Muenster, Muenster, Germany

Bacterial super-infections are a major complication in influenza diseases resulting in significant morbidity and mortality. Most of the fatal cases in the course of an influenza A virus (IAV) infection are a result of secondary pneumonia caused by different bacteria, among which *Staphylococcus aureus* (*S. aureus*) plays an important role.
One potent, highly regulated defense mechanism in response to invading microorganisms is the programmed cell death (apoptosis) that eliminates individual cells without inducing an inflammatory response. Unsurprisingly pathogens like IAV and *S. aureus* have evolved strategies to manipulate the host cell death machinery to increase their replication and survival. While, during ongoing infection, IAV-induced apoptosis facilitates the export of ribonucleoproteins out of the nucleus enhancing the release of progeny virions, *S. aureus* inhibits apoptosis via a significant increase in the expression of anti-apoptotic genes.
Although apoptosis is very well analysed during infections by either IAV or *S. aureus* alone, until today it is poorly understood how this process is controlled in presence of both pathogens. In this study we provide novel insights into the regulation of apoptotic mechanisms during simultaneous infection with IAV and *S. aureus*. Our data indicate that *S. aureus* is able to inhibit the IAV-induced apoptotic cellular response through decreased expression of the proapoptotic factor TRAIL resulting in a reduction of PARP cleavage. Thus, we unraveled a *S. aureus*-mediated mechanism that supports intracellular survival of the bacterium and may contribute to increased pathogenicity upon IAV and *S. aureus* super-infections.

Board No: 22 *Pathogen-Cell Interaction and Immunity*

CHICKEN EMBRYO AS A MODEL OF INFECTION IN BRUCELLOSIS

G. Wareth[1,2,3], D. Böttcher[4], M.C. Elschner[1], A. Ali Shehata[5], H.A Schoon[4], U. Roesler[3], H. Neubauer[1], F. Melzer[1]

[1]Institute of Bacterial Infections and Zoonoses, Friedrich Loeffler Institut, Federal Institute for Animal Health, Jena, Germany, [2]Department of Pathology, Faculty of Veterinary Medicine, Benha University, Qalyobia, Egypt, [3]Institute of Animal Hygiene and Environmental Health, Free University of Berlin, Berlin, Germany, [4]Institute of Pathology, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany, [5]Institute of Virology, Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany

Brucellae are intracellular stealthy pathogens causing disease in humans and in a wide range of animals either domestic or wild. The aim of the present work was to investigate the virulence of *Brucella microti* for chicken embryos in an *in ovo* infection model. Our study describes first results on the multiplication of *B. microti* in chicken embryos as well as gross and histopathology. Inoculation of *B. microti* with a dose of 1.6 E+03 bacteria through the allantoic and yolk sac routes provoked marked gross lesions i.e. hemorrhages and necroses. Mortality rate was 60% and 40% in days 3 and 4, respectively in allantoic sac route. In yolk sac route mortality rate was 40% in days 2 and 3, and was 20% in days 4 post inoculation. Re-isolation of *B. microti* proved rapid multiplication of bacteria (1.7 E+12 within 2 days). Apoptoses and necroses of single cells or groups of cells in liver, kidney, spleen, lung, gastrointestinal tract and chorioallantoic membrane were predominant histopathological lesions. Cytoarchitecture damages induced in these organs after inoculation of *B. microti* have demonstrated the proliferation and pathogenicity of *B. microti* for chicken embryos. Our results suggest that, even though chicken are no mammals, they are useful experimental animals to study the pathogenesis, pathogen interactions and immunopathology of brucellae.

Board No: 23 *Pathogen-Cell Interaction and Immunity*

EXPLORING NATURAL PRODUCTS TO BOOST THE INNATE IMMUNE SYSTEM AGAINST BACTERIAL INFECTIONS

R. Yaseen[1], W.N. Setzer[2], H.Y. Naim[1], M. von Köckritz-Blickwede[1]

[1]Department of Physiological Chemistry, University of Veterinary Medicine Hannover, Hannover, Germany , [2]Department of Chemistry, University of Alabama, Huntsville, United States

The emergence of antibiotic resistant microorganisms poses great challenges to the human and veterinary medicine. An alternative approach for the treatment of difficult infections, such as those involving antimicrobial resistance or compromised host immunity could be the pharmacological enhancement of the antimicrobial capabilities of phagocytes. Pharmacological agents which boost the host immune system could conceivably be used alongside conventional antibiotic treatment for successful therapy of the infection. The goal of this study is to search for novel natural products with the ability to boost the host immune defence against bacterial infections.

Here, we have screened a series of tropical rainforest plant extracts for their ability to boost the antimicrobial activity of blood and host immune cells. A crude acetone extract of bark from Byrsonima crassifolia (BYCRBA) was identified as interesting candidate. BYCRBA is a medicinal tree that ranges from Mexico south to Peru and Paraguay as well as the Caribbean. The aim of this study was to characterize the effect of BYCRBA on the antimicrobial activity of the innate immune system. Therefore, human and bovine fresh blood or blood-derived monocytes were treated with BYCRBA and then infected with *Staphylococcus aureus*. The growth of S. aureus was monitored and revealed that BYCRBA barc extracts exhibited no direct antimicrobial effect against the bacteria. However, interestingly, we found that BYCRBA is able to boost the antimicrobial activities of human blood and blood-derived monocytes against S. aureus. Vitexine, which was recently identified as a flavonoide-component of this plant extract showed similar results. Our study lead to the conclusion that BYCRBA bark extracts might have a beneficial effect on the host innate immune system by boosting the antimicrobial capacities of blood-derived cells as the first line of defense against invading pathogens. Current experiments focus on the underlying biochemical mechanisms associated with this phenomenon.

Finally, this project might help to identify new therapeutic targets based on natural products, which can be further developed as new therapeutic treatment strategies against bacterial infections in human as well as animals.

Board No: 24 *Pathogen-Cell Interaction and Immunity*

A COMPREHENSIVE ANALYSIS OF THE PRIMARY TRANSCRIPTOME OF THE *ESCHERICHIA COLI* O104:H4 PAA PLASMID REVEALS NEW INSIGHTS INTO VIRULENCE GENE REGULATION

P. Zhelyazkova[1], K. Förstner[2,3], C.M. Sharma[3], J. Vogel[2], H. Karch[1], A. Mellmann[1]

[1]Institute of Hygiene, University of Münster, Münster, Germany, [2]Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany, [3]Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany

In 2011 *Escherichia coli* O104:H4 (*E. coli* O104:H4) caused the largest outbreak of foodborne illness in Germany. More than 3000 people were infected, and of these more than 800 were diagnosed with hemolytic uremic syndrome (HUS). *E. coli* O104:H4 is a highly virulent hybrid strain carrying characteristic features of both enterohemorrhagic *E. coli* (e.g. a chromosomally integrated Shiga toxin 2-encoding bacteriophage) and enteroaggregative *E. coli* (e.g. a pAA plasmid, coding for aggregative adherence fimbriae I, AAF/I, cluster). A main focus of our research is to gain further insight into the virulence factors and mechanisms contributing to the exceptional pathogenicity of *E. coli* O104:H4. Our group recently showed that the outbreak strain can lose pAA during the course of illness and that this loss is associated with a significantly reduced progression to HUS (Zhang et al., 2013). This observation clearly underlines the importance of pAA in host-pathogen interaction and disease severity. Here, we analyzed the primary transcriptome of pAA of the clinical *E. coli* O104:H4 isolate LB226692 using differential RNA-seq (dRNA-seq), a recently established method that allows for the high throughput mapping of transcription start sites (TSS) and non-coding RNAs. dRNA-seq includes an enzymatic step prior to RNA-seq, in which Terminator exonuclease (TEX) is used to degrade RNAs with a 5′ monophosphate (i.e. processed transcripts), but not with a 5′ triphosphate structure (i.e. primary transcripts). The comparison of cDNA libraries generated from TEX untreated and TEX treated RNA can therefore be exploited to identify protected primary transcripts and their TSS. The dRNA-seq analysis revealed a surprising complexity of the pAA primary transcriptome and of the overall transcriptional organization of virulence-associated genes. We have detected numerous TSS within the operon coding for the AAF/I, allowing for transcriptional uncoupling of this polycistronic unit and differential regulation of the individual fimbria components. Moreover, we mapped highly abundant/multiple antisense transcripts to genes involved in intestinal colonization and inflammation (e.g. *aap* and *sepA*), suggesting a role of asRNA in the posttranscriptional regulation of these virulence genes. Last but not least, we mapped many TSS in intergenic regions which could function as virulence associated trans-encoded ncRNAs.

Board No: 25 *Pathogen-Cell Interaction and Immunity*

ISOLATION OF A NOVEL NODAVIRUS WITH NOVEL FEATURES: MOSINOVIRUS EXPRESSES TWO SUBGENOMIC RNAS, ENCODES A CAPSID GENE OF UNKNOWN ORIGIN AND A SUPPRESSOR OF THE ANTIVIRAL RNAI PATHWAY

F. Zirkel[1], S. Schuster[2], A. Kurth[3], K.W.R. van Cleef[2], C. Drosten[1], R.P. van Rij[2], S. Junglen[1]

[1]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [2]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Radboud Institute of Molecular Life Sciences, Nijmegen, Netherlands, [3]Center for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany

The identification of novel viruses provides important information about virus evolution and diversity. Here, we discovered a unique novel virus, named Mosinovirus (MoNV), in mosquitoes from a tropical rainforest region in Côte d'Ivoire. MoNV was isolated in C6/36 (*Aedes albopictus*) cells and induced strong cytopathic effects. The entire MoNV genome was sequenced by deep sequencing and RACE PCR. The MoNV genome consists of two segments of positive-sense RNA of 2,972 nt (RNA 1) and 1,801 nt (RNA 2) in length. Its putative RNA-dependent RNA polymerase is encoded on RNA 1 and shares 43% pairwise amino acid identity with its closest relative, Pariacoto virus, an *Alphanodavirus*, family *Nodaviridae*. Nodaviruses have a bisegmented genome and infect insects, fish, nematodes and prawns. Unexpectedly, the putative capsid protein encoded on RNA 2 showed a maximal pairwise identity of only 16% to Lake Sinai virus, an unclassified virus with a non-segmented genome that was identified in honey bees. Moreover, MoNV virions are non-enveloped and are about 50 nm in diameter, larger than any of the known nodaviruses. Mature virions contain a ~56 kDa capsid protein. Northern blot analyses showed that MoNV expresses two subgenomic RNAs of 580 nt (RNA 3) and 292 nt (RNA 4) from segment 1. RNA 4 encodes a viral suppressor of RNAi that has a similar function as the B2 protein of other nodaviruses, despite lacking any recognizable similarity to these proteins. MoNV B2 is able to bind long dsRNA as well as 21-nt small interfering RNA (siRNA) and, accordingly, it inhibits Dicer-2 mediated processing of dsRNA into siRNAs. Phylogenetic analyses based on the RdRp and on the capsid proteins differed in their topology. In phylogenetic analyses based on the RdRp protein sequences, MoNV grouped together with Pariacoto virus and other unclassified nodaviruses. However, in phylogenetic analyses based on the capsid protein sequences MoNV branched from a deep node in distant relationship to nodaviruses and Lake Sinai virus. In summary, we have identified a novel member of the family *Nodaviridae* that acquired its capsid gene via reassortment from an unknown, distantly related virus beyond family level.

Board No: 26 *Pathogen-Cell Interaction and Immunity*

IDENTIFICATION OF DETERMINANTS IN TYPE II TRANSMEMBRANE SERINE PROTEASES WHICH CONTROL THE ACTIVATION OF INFLUENZA A VIRUSES

P. Zmora, A.S. Moldenhauer, S. Pöhlmann

Infection Biology Unit, German Primate Center, Göttingen, Germany

Each year influenza virus infection causes 3-5 million cases of severe illness and 250.000-500.000 deaths worldwide. Proteolytic cleavage of the influenza virus hemagglutinin (HA) by host cell proteases is essential for viral infectivity and the responsible proteases are potential targets for antiviral intervention. Recently, members of the type II transmembrane serine protease (TTSP) family, including TMPRSS2 and HAT, were shown to activate influenza viruses. However, the TTSP family comprises approximately 20 proteins and it is unclear why some have the ability to activate HA while others do not. Therefore, the aim of this study was to identify domains in TTSPs, which control the ability of these enzymes to cleave and activate HA.

We have created and functionally analysed chimeras between TMPRSS2, which activates HA, and TMPRSS3, which fails to activate HA. For this, the cytoplasmic, transmembrane, stem or catalytic domains were exchanged between these proteins. We demonstrate that the exchange of the stem region abrogates HA activation by TMPRSS2 and endows TMPRSS3 with the ability to activate HA, indicating a key role of the stem in HA activation. Immunostaining and confocal microscopy revealed that the active TMPRSS3 chimera colocalized with HA while the wt protein did not. Moreover, the HA-activating TTSPs TMPRSS2, DESC1 and MSPL highly colocalized with HA while the inactive TTSPs TMPRSS3 and TMPRSS10 did not, indicating that the cellular localization of TTSPs might be an important determinant of HA proteolytic activation. These results indicate that the stem region in TTSPs can determine HA activation, potentially by controlling the intracellular localization of these enzymes.

Board No: 27 *Reservoirs of Zoonoses*

MOLECULAR SURVEY AND GENETIC CHARACTERIZATION OF *ANAPLASMA PHAGOCYTOPHILUM* IN SMALL RUMINANTS HOSTS OF *RHIPICEPHALUS* TICKS FROM TUNISIA

M. Ben Said[1], H. Belkahia[1], M. Bousrih[1], M. Yahiaoui[1], M. Gharbi[2], M. Daaloul[1], M.A. Darghouth[2], L. Messadi[1]

[1]Laboratory of Microbiology, National School of Veterinary Medicine of Sidi Thabet, Tunisia., Ariana, Tunisia, [2]Laboratory of Parasitology, National School of Veterinary Medicine of Sidi Thabet, Tunisia, Ariana, Tunisia

Anaplasma phagocytophilum, an obligate intracellular bacterium, is the etiological agent of granulocytic anaplasmosis described in several species including humans. Although small ruminants have been implicated as important hosts for transmission of granulocytic anaplasmosis, no epidemiologic investigation of small ruminant infections in Tunisia has been conducted.

The aims of this study were to estimate the molecular prevalence of *A. phagocytophilum* in goats (n = 303) and sheep (n = 260) from northern Tunisia and to characterize by partial 16SrRNA gene sequencing the genotypes of this rickettsia.

The molecular prevalence of *A. phagocytophilum* was 47.5% and 7.7% in goats and sheep respectively. In goats, prevalence rates were estimated at 48.9 and 43.0% in Bizerte and Beja governorates respectively. The infection prevalence varied significantly according to farms and age of males. In sheep, prevalence rates were estimated at 2.9 and 13.3% in El Alia and Khetmine localities (belonging both to the governorate of Bizerte) respectively. *A. phagocytophilum* infection prevalence varied significantly according to farms and tick infestation.

Regarding the parasitological survey, a total of 919 ticks collected from sheep belonged to *Rhipicephalus turanicus* (52.8%), *R. sanguineus* (44.0%) and *R. annulatus* (3.3%) and 304 ticks collected from goats belonged to *R. turanicus* (79.9%), *R. bursa* (14.5%), *R. sanguineus* (4.9%) and *Hyalomma excavatum* (0.7%). *R. turanicus* was the most abundant tick species with a value of 4.14 and 2.47 ticks per sheep and goat, respectively.

The analysis of *A. phagocytophilum* 16SrRNA sequences revealed novel genotypes. Phylogenetic study of 16SrRNA sequences of *A. phagocytophilum* indicated that the *A. phagocytophilum*genotypes from sheep differed from the genotypes of goats in the investigated localities.

The present work is the first published report of *A. phagocytophilum* infection in small ruminants from Tunisia. The results may promote the understanding of the epidemiological status of these pathogens in Tunisia and contribute to the construction of models of risk prediction for tick-borne diseases in the country.

Board No: 28 *Reservoirs of Zoonoses*

***BORRELIA BURGDORFERI SENSU LATO* IN RUMINANTS FROM TUNISIA, PRELIMINARY DATA**

M. Ben Said, H. Belkahia, M. Bousrih, M. Yahiaoui, Z. Issaoui, D. Hattab, M. Daaloul, L. Messadi

Laboratory of Microbiology, National School of Veterinary Medicine of Sidi Thabet, Ariana, Tunisia

Borrelia burgdorferi sensu lato (s.l.) are tick-transmitted spirochaetes. Among currently known 19 genospecies, seven of them are known or considered causative agents of Lyme borreliosis, namely *B. afzelii*, *B. garinii*, *B. bavariensis*, *B. bissettii*, *B. burgdorferi* sensu stricto, *B. spielmanii* and *B. valaisiana*. The animal reservoirs of *Borrelia burgdorferi* s.l. are numerous, mainly wild small mammals and birds. Ruminants have not been enough investigated.

The aim of this study was to investigate the prevalence of *B. burgdorferi* s.l. infection among healthy ruminants in Tunisia. A total of 303 goats, 260 sheep, 232 cattle and 226 dromedaries were collected for real time PCR targeting *B. burgdorferi* s.l. 23S rRNA gene. Real time PCR-positive rates were 30.40% for goats, 6.15% for sheep, 1.29% for cattle, and 1.7% for dromedaries.

In goats, the prevalence of *B. burgdorferi* s.l. varied significantly according to bioclimatic zone, age of females and tick infestation. The highest prevalence of *B. burgdorferi* s.l. was found in Joumine (51.9%) (Governorate of Bizerte), a humid area while the lowest was reported in El Alia (1.4%) (Governorate of Bizerte), a sub-humid area. In sheep, the prevalence rate varied significantly according to age of females and breed. The highest prevalence of *B. burgdorferi* s.l. was found in Khetmine (10.0%) (Governorate of Bizerte), while the lowest was reported in El Alia (2.6%) (Governorate of Bizerte). In cattle, the three positive samples were collected from adult females (≥ 3 years) located in sub-humid bioclimatic zone. In dromedaries, the four positive samples were collected from two adult males (≥ 5 years) situated in semi-arid area and two adult females (≥ 6 years) located in arid area.

These preliminary results indicated that the emerging tick-borne *Borrelia burgdorferi* s.l. infection is already prevalent in Tunisia and ruminants, especially goats, could be important hosts for the transmission of the *Borrelia burgdorferi* s.l. complex infection. The present work is the first report providing molecular detection of *Borrelia burgdorferi* s.l. infection in goats and dromedaries. Further studies are necessary to identify and characterize of all *Borrelia burgdorferi* s.l. genospecies infected these ruminants.

Board No: 29 *Reservoirs of Zoonoses*

FELINE VECTOR BORNE DISEASES OF ZOONOTIC CONCERN IN DOMESTIC AND STRAY CATS IN GREECE

C.N. Tsokana[1], A. Giannakopoulos[1], P. Pezaros[2], A. Kromydas[2], I. Samoladas[2], M. Svanias[2], E. Skoutzou[2], Z. Zarra[2], I. Diamantis[2], A. Lemas[2], D. Balomenos[2], G. Ntavaras[2], I. Pasxalidis[2], T. Georgiadis[2], G. Kaltsas[2], V.I. Kontos[3], V. Spyrou[4], L.V. Athanasiou[5], A.R. Burriel[1], C. Billinis[1]

[1]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece, [2]Companion Animal Private Clinic, Attiki, Greece, [3]Department of Veterinary Public Health, National School of Public Health, Athens, Greece, [4]Department of Animal Production, Technological Education Institute of Larissa, Larissa, Greece, [5]Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece

Cats and dogs may act as reservoirs, amplifying hosts or sentinels of a wide range of pathogens transmitted by arthropods thus involving in the transmission cycle of some agents of vector borne diseases [1]. Leishmaniasis has been described in cats since 1912. Cats are considered to be a secondary reservoir host of *L. infantum* [2-4]. The potential role of cats in the epidemiology of leishmaniosis, becomes even more complex because only repellents effective against sand flies, the pyrethroids, are toxic to cats making thus difficult the prevention of feline *Leishmania* infection [1]. Additionally, cats are the major reservoir of *Bartonella henselae*, *B. clarridgeiae* and *B. koehlerae* which are transmitted to humans, while they may function as accidental host of *B. quintana*, *B. bovis* and *B. vinsonii subsp. berkhoffii.* Subclinical infection with B. clarridgeiae or B. henselae, the agents of the cat scratch disease, is frequently reported in cats [5].

The aim of the present work was to molecularly detect two feline vector-borne pathogens with zoonotic importance. We herein report the preliminary results of our ongoing study in the context of the research funding program THALES, concerning the occurrence of *L. infantum* and *B.henselae* infection in owned and stray cats. Thus far, 79 blood and serum samples have been collected from cats in Thessaly and Attiki, Greece. The blood samples have been examined by PCR and the serum samples have been examined by IFAT for the detection of DNA and antibodies against *L.infantum*and *B.henselae* respectively. The prevalence was found to be 5% (4/79) and 7.5% (6/79) respectively.

In recent years, feline vector borne diseases present wider geographic distribution and increased global prevalence. This fact may be attributed to environmental, demographic and human behavioral factors along with the direct impact of climate changes on the abundance, the geographical distribution and the vectorial capacity of vector arthropods [1]. The above mentioned have contributed to the changing epidemiology of these arthropod-borne diseases making thus their epidemiological investigation, the alert of the veterinary community, the owners and the public health authorities for the risk of transmission of vector-borne pathogens, a necessity.

References:

[1] Maia C, Ramos C, Coimbra M, Bastos F, Martins Â, Pinto P, et al. Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. Parasit Vectors 2014;7:115

[2] Gramiccia, M., 2011. Recent advances in leishmaniosis in pet animals: epidemiology, diagnostics and anti-vectorial prophylaxis. Vet. Parasitol. 181, 23–30. doi:10.1016/j.vetpar.2011.04.019

[3] Maroli, M., Pennisi, M.G., Di Muccio, T., Khoury, C., Gradoni, L., Gramiccia, M., 2007. Infection of sandflies by a cat naturally infected with Leishmania infantum. Vet. Parasitol. 145, 357–360

[4] Poli, A., Abramo, F., Barsotti, P., Leva, S., Gramiccia, M., Ludovisi, A., Mancianti, F., 2002. Feline leishmaniosis due to Leishmania infantum in Italy. Vet. Parasitol. 106, 181–191.

[5] Athanasiou LV, Chatzis MK, Kontou IV, Kontos VI, Spyrou V 2012. Feline bartonellosis. A review. J Hellenic Vet Med Soc 63(1): 63-73

Acknowledgements:

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

Board No: 30 *Reservoirs of Zoonoses*

TRICHINELLA INFECTION IN WILD ANIMALS OF UKRAINE

Y. Didyk

Acarology, Schmalhausen Institute of Zoology Nas of Ukraine, Kyiv, Ukraine

Trichinellosis is one of the most dangerous diseases common to humans and animals. It is caused by nematodes from the genus *Trichinella* Railliet, 1895. Humans get parasite through the consumption of raw or undercooked meat with larvae of *Trichinella*. In Ukraine infection was detected in domestic pigs and humans. Infected pork was the main source of human *Trichinella*infection in the last century. But today in Ukraine the most cases of human Trichinellosis is caused by the consumption of infected game (wild boars, badgers etc.). The aim of this work was to study the *Trichinella* prevalence and species composition in wild animals.

Materials were collected during the hunting seasons in 2002-2013 years. Ungulates and carnivores were studied for the presence of *Trichinella*. The samples were examined following the standard protocol of ITRC. The parasite larvae isolated from infected animals were identified by multiplex PCR analyses (Borsuk et al., 2003; Pozio and La Rosa, 2003).

Trichinella was found in all regions of Ukraine. Larvae were detected in 3% investigated wild boars (6), 15.4% wolves (12), 15.8% red foxes (75), 12% martens (6), 10% badgers (2). Wolves and foxes are the main reservoir of *Trichinella* in a sylvatic cycle.

The three species of *Trichinella* were detected in Ukraine. *T. britovi* was found in almost all infected wild animals. *T. nativa* was found only in foxes in Northern Ukraine. *T. spiralis* and mixed infection *T. britovi*-*T. spiralis* was found in wolves in Southern Ukraine. *T. britovi* (found in the majority of infected wolves and red foxes) is a dominant species in the sylvatic cycle in Ukraine.

The infestation extent of wild mammals' trichinellosis has increased over the past 30 years in Ukraine: 3.5% — first half of 1970-s, 8.8% – 1980-s and 15% (our data). Probably, it's caused by high densities of predators' populations that correspondingly lead to increased levels of scavenging and cannibalism, and also humans have big influence on *Trichinella* prevalence. In Ukraine hunters leave animal carcasses in forests like "baits" or throw it away as garbage near villages. All these increase *Trichinella* prevalence amongst wild animals in sylvatic cycles and make a risk for infection animals in domestic cycles and for humans.

Board No: 31 *Reservoirs of Zoonoses*

EPITHELIAL CELL LINES FROM BATS, RODENTS AND INSECTIVORES – A NOVEL TOOL FOR *IN VITRO* INVESTIGATION OF PATHOGEN-HOST INTERACTION

I. Eckerle[1], R. Ulrich[2], A. Rang[3], B. Klempa[3,4], L. Radosa[3,4], M.A. Müller[1], C. Drosten[1]

[1]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [2]Institute for Novel and Emerging Infectious Diseases, Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Greifswald Isle of Riems, Germany, [3]Institute of Virology, Charité, Berlin, Germany, [4]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Despite the great interest created by emerging zoonotic viruses, there is still a lack of *in vitro* models that adequately reflect the exclusive virus-host adaptation of most zoonotic viruses. While the majority of species involved in zoonotic transmission are not available as laboratory animals due to their conservational status or the inability to breed them in captivity, cell lines derived from those species can serve as an acceptable surrogate to study zoonotic viruses in the natural host context.

We have recently established a broad range of reservoir-derived cell lines from bats, providing important insight into immunological and reservoir-host specific mechanisms of zoonotic viruses. However, there has been no focus to selectively culture epithelial cells from zoonotic reservoir hosts so far. The epithelia of the respiratory and renal tract are the predominantly involved cell types in terms of virus entry, replication and shedding. During airborne transmission, it is the first tissue encountered by viral particles and therefore serves as an important barrier of inter-species transmission.

Here, we present an algorithm for establishment and characterization of epithelial cell lines derived from trachea and kidney samples of small mammals. The approach focuses on generation of primary cells derived from samples collected in the field in order to cover a broad range of important reservoir species, including those species that cannot be held in captivity.

Part of this work is funded by the German Research Platform for Zoonoses in the interdisciplinary cross-sectional project "EpiZell". First results of the project include the establishment of epithelial cell lines from important hantavirus reservoir hosts such as *Myodes glareolus*, *Apodemus agrarius* and *Apodemus flavicollis*. Cells were successfully cultured under standardized conditions from both fresh and frozen organ specimens and immortalized for the generation of permanent cell lines. Virus infections studies showed susceptibility and efficient replication of zoonotic viruses, including those that are associated with the respective species in the field.

Board No: 32 *Reservoirs of Zoonoses*

MIGRATORY BIRDS HARBOR HIGH NUMBER OF ESBL-PRODUCING *E. COLI* IN PAKISTAN

M.M. Gilani, S. Raza, F. Sarwar, A. Umer Khan, I. Hussain

Institute of Microbiology, University of Agriculture, Faisalabad, Faisalabad, Pakistan

Background: Extended spectrum beta-lactamases (ESBLs) producing bacteria have become major concern in humans and veterinary medicine due to their resistance to third generation cephalosporin antibiotics. Migratory birds could be the reservoirs and potential source of spread of ESBLs producing *E. coli*. In the present study, we focused on the prevalence of ESBLs producing *E. coli* in migratory avian species, which arrives in Pakistan from Siberia and Central Russia via international migratory bird-route number 4 or Indus flyway.

Methods: A total of 100 migratory birds fecal swabs were collected during October 2013 to February 2014 in Pakistan. These birds mainly include mallard, Eurasian coot, starlings, common pochard, shovler duck, gadwell ducks, quail, red-headed pochard and wigeon duck. The samples were screened for ESBL by cultivation on CHROMagar-ESBL (CHROMagar, France). All *E. coli* isolates were phenotypically confirmed as ESBL using antibiotic sensitivity testing and double disk synergy tests according to CLSI criteria. All isolates were biochemically confirmed as *E. coli* using commercial Remel RapID ONE test (Remel, UK). PCR was used to confirm the presence of *bla*CTX-M, *bla*TEM, and *bla*SHV genes among ESBL- *E. coli* isolates.

Results: A total of 40 out of 100 (40%) migratory birds carried ESBL *E. coli*. Highest number of ESBL producing *E. coli* were found in mallard n=15 (37.5%) followed by Eurasian coot n=7 (17.5%), common pochard n=6 (15%), starling n=3 (7.5%), Shovler duck n=2 (5%), gadwell duck n=2 (5%), red head pochard n=2 (5%), wigeon duck n=2 (5%) and quail n=1 (2.5%). PCR showed *bla*TEM as the most frequent ESBL type 50% (20/40) followed by *bla*CTX-M 23% (9/40) whereas *bla*SHV was found only in one *E. coli.* Out of 9 *bla*CTX-M isolates, eight also harbor *bla*TEM gene.

Conclusion: This is the first report in Pakistan showing migratory birds as reservoirs of ESBL producing *E. coli.* The significantly high rates of ESBL *E. coli* in migratory birds could be a potential source of zoonosis and transboundary spread of ESBL *E. coli.* Further studies are underway to unravel phylogenetically important clone types among ESBL *E. coli*.

Board No: 33 *Reservoirs of Zoonoses*

BACTERIAL PATHOGENS (*BORRELIA* AND *ANAPLASMA*) INFECTING VARIOUS REPTILE SPECIES

B. Haklová[1], A. Hižňanová[2], I. Majláth[1,2], G. Földvári[3], P. Tryjanowski[4], N. Kokošová[2], V. Petrilla[5], V. Majláthová[1]

[1]Department of Vector Borne Diseases, Institute of Parasitology Sas, Hlinkova 3, Košice, Slovakia, [2]Institute of Biology and Ecology, University of P. J. Šafárik in Košice, Moyzesova 11, Košice, Slovakia, [3]Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary, [4]Institute of Zoology, Poznan University of Life Sciences, Poznań, Poland, [5]Department of Anatomy, Histology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, Košice, Slovakia

Reptiles are hosts for different species of ectoparasites, which can transmit wide spectrum of pathogens. *B. burgdorferi* sensu lato (s.l.) complex consist of at least 19 genospecies, including *B. lusitaniae*, associated with lizards. Recently, new group of reptile-associated *Borrelia* (REP) was identified from imported reptiles and their ectoparasites. The role of reptiles in the transmission of *Anaplasma* was not described yet; we have only very few information about the presence of these bacteria in reptiles. We investigated the presence of abovementioned bacteria in various reptile species. Blood from reptiles was taken via a ventral puncture of the vena coccygea. Ticks and skin biopsy (tail tip, liver or spleen) specimens were removed from reptiles by sterile implements and stored in 70% ethanol. Samples were tested by PCR method and analyzed by RFLP and SSCP methods. A total of 624 reptiles belonging to 41 genera from Europe (Slovakia, Poland, Hungary, Romania, Sweden and Greece) and from exotic countries (Africa, Asia and America) were examined. Bacteria from *B. burgdorferi* s.l. complex were detected in 296 skin biopsy specimens of lizard species from Europe (15.2%). Predominantly detected genospecies was *B. lusitaniae*, although *B. valaisiana* was also found in *Lacerta viridis* from Slovakia. A total of 572 *Ixodes ricinus* ticks (385 larvae and 187 nymphs) from reptiles from Europe were tested, with 12.4% individuals positive. *B. lusitaniae* was the most often detected genospecies, although other genospecies, *B. burgdorferi* sensu stricto, *B. valaisiana*, *B. garinii* and *B. afzelii*, were also present. REP *Borrelia* was detected in *Python sebae natalensis* and *B. burgdorferi* sensu stricto in *Morelia viridis*. Species from the family Anaplasmataceae was found in 31.4% of all individuals tested. PCR-SSCP method revealed the existence of undescribed *Anaplasma* sp. in reptiles from Europe (*L. viridis*, *L. agilis* and *Natrix natrix*) and from exotic countries (*P. sebae natalensis* and *M. capensis*).

The study was supported by the project of Research & Development Operational Programme funded by the ERDF (code ITMS: 26220220116) (0,5) and by VEGA 1/0417/14. This study was conducted under the frame of the EurNegVec COST Action TD1303.

Board No: 34 *Reservoirs of Zoonoses*

HUMORAL RESPONSE OF CATS TO THE EXPERIMENTAL INFECTION WITH THE DIFFERENT CLONAL TYPES OF *TOXOPLASMA GONDII*

M. Hosseininejad[1], H. Moshtaghi[2], F. Hosseini[1], N. Eshraghi Samani[1]

[1]Veterinary Clinical Sciences, University of Shahrekord, Shahrekord, Islamic Republic of Iran, [2]Food Hygiene and Quality Control, University of Shahrekord, Shahrekord, Islamic Republic of Iran

Toxoplasma gondii is one of the most prevalent parasitic infections in world. Rh, NED and Me49 are of the most prevalent clonal type of the parasite isolated till now. Differences in pathogenicity and virulence of different types have been investigated in different studies. No controlled study was performed to compare ability of different types to initiate humoral immune response. We investigated IgG antibody responses of kittens infected with each of these three clonal types. No antibodies were detectable at least until 7 days post infection for types Rh and NED while this period of no response was 19 days for ME49. Serum ELISA indices were significantly higher in kittens infected with Rh and NED tpes in compare to ME49.

Board No: 35 *Reservoirs of Zoonoses*

VIROME ANALYSIS OF EUROPEAN BATS: A TOOL TO CORRELATE HISTO-PATHOLOGICAL CHANGES IN ORGAN TISSUE TO INFECTIOUS DISEASES?

C. Kohl[1], A. Brinkmann[1], P.W. Dabrowski[1], M. Wegener[1], A. Radonić[1], K. Mühldorfer[2], A. Nitsche[1], G. Wibbelt[2], A. Kurth[1]

[1]Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany, [2]Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

Viruses responsible for disease outbreaks in humans emerge either from the human population or by spill-over from animal hosts. Sixty per cent of emerging viruses have a zoonotic origin and therefore are a major threat to public health. Whereas viruses in domestic animals are well studied, viruses of wildlife are still rarely examined. Since most recent disease outbreaks are associated with zoonotic transmission events of newly emerging viruses, it has become evident that surveillance and evaluation of viruses prevalent in wildlife is of particular importance. Attention should be paid to animals which are increasingly recognized as potential reservoir hosts (e.g. bats). Bats are widely distributed throughout the world, inhabit rural as well as urban environments and share very unique immunological features. Many viruses exist alongside their hosts in symbiotic mutualism and this is particularly assumed for bat viruses. Whether viruses are capable of causing a disease in their bat-host is a question we would like to address here. In this study, 375 moribund or dead-found microchiropteran bats from throughout Germany have been examined. More than thirty per cent showed potentially virus-related histo-pathological changes of the internal organs. A novel method was established and extensively evaluated for the purification and enrichment of known and unknown viruses directly from virus-infected organ tissue. Subsequently, bat-organs have been pooled and purified for deep sequencing of the virome. With this approach, novel viruses belonging to different virus families and genera were detected.

Board No: 36 *Reservoirs of Zoonoses*

COMPARATIVE METAGENOMIC PROFILING OF SYMBIOTIC BACTERIAL COMMUNITIES ASSOCIATED WITH IXODES PERSULCATUS, IXODES PAVLOVSKYI AND DERMACENTOR RETICULATUS TICKS

A. Kurilshikov[1], N. Livanova[1], N. Fomenko[1], V. Rar[1], M. Kabilov[2], N.V. Tikunova[1]

[1]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russian Federation, [2]SB RAS Genomics Core Facilty, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russian Federation

Ticks are the vectors of many infectious agents important to animal and human health. The most epidemiologically significant tick species in Siberia and the Far East are *Ixodes persulcatus* and *I. pavlovskyi* transmitting a large number of bacterial and viral pathogens. Another tick species of the family *Ixodidae*, *Dermacentor reticulatus,* is involved in the transfer of some infectious agents, as well. Despite the great epidemiological importance of these tick species, their microbial communities are still little investigated.
Bacterial communities associated with *I.persulcatus*, *I.pavlovskyi* and *D.reticulatus* ticks were evaluated using high throughput sequencing of V3-V5 16S rRNA amplicon libraries.
Ticks were collected in Novosibirsk region, Western Siberia. Total DNA was extracted from washed ticks and two pools consisting of DNA from ~ 100 males and two - from ~ 100 females were randomly formed for each species. Sequencing of twelve 16S rRNA amplicon libraries was performed using Illumina Miseq. Data analysis was performed using QIIME toolkit v.1.7.0 and R language v.3.0.0. First, reads were clustered with uclust package with 97% similarity cutoff, then obtained OTUs were classified with RDP classifier using cutoff of 0.85. Downstream statistical analysis was made with R packages "vegan" and "ade4".
The taxonomical composition of the twelve obtained datasets showed *Alpha-* and *Gammaproteobacteria* as dominant bacterial classes. The most common genera were *Acinetobacter* (31,4%),*Rickettsia* (20,5%) and *Francisella* (16,7%). However, distribution of genera differed for each tick species. Thus, the percentage of bacteria belonging to *Rickettsiales* order was the highest in*I.persulcatus* (76%, females and 41%, males) but the lowest in *I.pavlovskyi* ticks (less than 4%) in spite of the genetic closeness of these species.

Bacteria from the genus *Francisella* were dominant in *D.reticulatus* ticks but extremely rare in two other tick species. Pathogenic *Borrelia* spp (*B.burgdorferi* s.l. and *B. miyamotoi*) were found in *I.persulcatus* and *I.pavlovskyi* ticks only. Pathogenic bacteria from the family *Anaplasmataceae* including *Anaplasma phagocytophilum, Ehrlichia muris* and «*Candidatus* Neoehrlichia mikurensis» were detected in all three species. In general, the diversity was significantly higher in *I.pavlovskyi* ticks; the number of genera varied from less than 50 for *D.reticulatus* ticks to more than 90 for *I.pavlovskyi* ticks.

Board No: 37 *Reservoirs of Zoonoses*

LOCATION OF BLACTX-M-1 AND QNRS1 GENES ON INCN PLASMIDS FROM CLINICAL *ESCHERICHIA COLI* ISOLATES COLLECTED FROM DISEASED ANIMALS

G. Michael Brenner[1], K. Kadlec[1], H. Kaspar[2], S. Schwarz[1]

[1]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (), Neustadt Mariensee, Germany, [2]Monitoring of Resistance to Antibiotics, Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

Broad host range plasmids of the incompatibility group N (IncN) play an important role in the dissemination of the extended-spectrum β-lactamase (ESBL) gene *bla*$_{CTX-M-1}$ and plasmid-mediated quinolone resistance (PMQR) genes in *Escherichia coli*. The aim of this study was to investigate *bla*$_{CTX-M-1}$-carrying IncN plasmids among *E. coli* isolates from diseased food-producing animals.

From a total of 531 *E. coli* isolates of animals suffering from enteric infections, collected in the German national resistance monitoring program GERM-Vet during 2008, 23/198 bovine and 17/333 porcine isolates were ESBL producers. Isolates carrying the *bla*$_{CTX-M-1}$ gene on IncN plasmids and the respective plasmids were characterized by: susceptibility testing to 28 antimicrobial agents, XbaI-macrorestriction analysis, electrotransformation experiments, PCR assays for detection of resistance genes and for the determination of the phylogenetic group and the replicon type, sequencing of *bla*$_{CTX-M-1}$ and *qnrS* genes, S1 nuclease PFGE and plasmid restriction analysis.

IncN plasmids encoding CTX-M-1 were found in 5/23 bovine and 8/17 porcine ESBL-producing isolates. XbaI-macrorestriction patterns showed no clonal relationship among 11 isolates, while the remaining two were closely related. The IncN plasmids exhibited similar sizes (35 to 50 kb), except for one large plasmid (ca. 145 kb) of a porcine isolate of the phylogenetic group D. Among the 13 IncN plasmids, eight PvuII restriction patterns were seen. Two patterns were shared by plasmids of bovine and porcine isolates. On a 50-kb plasmid, the PMQR gene *qnrS1*, which confers reduced susceptibility to fluoroquinolones, was found. The 145-kb plasmid harboured also genes for resistance to non-beta-lactam antibiotics: *aph(3')Ia* (kanamycin resistance), *tet*(A) (tetracycline resistance), *dfrA*(trimethoprim resistance) and *sul3* (sulphonamide resistance).

The detection of unrelated ESBL-producing *E. coli* isolates harbouring indistinguishable or similar IncN plasmids points towards a horizontal dissemination of these plasmids among *E. coli* from cattle and swine. The presence of additional resistance determinants on the ESBL-carrying plasmids indicates that co-selection of ESBL genes may occur even in the absence of beta-lactam antibiotics. Moreover, the co-location of ESBL and PMQR genes on broad host range plasmids may lead to an increase in treatment failure in animals and humans when any of these antimicrobial agents are used.

Board No: 38 *Reservoirs of Zoonoses*

EPIZOOTIOLOGICAL STUDIES IN POPULATIONS OF BATS ON THE SLOVAK REPUBLIC TERRITORY

A. Ondrejková, Ľ. Korytár, R. Ondrejka, M. Prokeš, P. Čechvala, Z. Hurníková

University of Veterinary Medicine and Pharmacy in Košice, Department of Epizootology and Parasitology, Košice, Slovakia

Bats are commoly encountered in Slovakia where they use a variety of natural and seminatural habitats throughout the year. It has been proven that 28 species of bats from two families (*Rhinolophidae* and *Vespertilionidae*) occur in the Slovak Republic territory. The chiropterofauna is an important component of ecosystem in various types of landscape. The study was carried out in the years 2007-2013. Species mosaic consisting of the common pipistrelle (*Pipistrellus pipistrellus*), the soprano pipistrelle (*Pipistrellus pygmaeus*), the serotine (Eptesicus serotinus), the noctule (*Nyctalus noctula*) and pari-coloured bat (*Vespertilio murinus*) were recorded. The public health risk in connection with potential European bat lyssavirus (EBLV) infection in bats was studied. Blood was collected for serologic survey on EBLV, the blood was taken by puncture of the antebrachial vein using mechanical micropipette. Rapid fluorescent focus inhibition test (RFFIT) according to Smith et al. (1973) was used for the detection of specific rabies antibodies in the bats serums. No serological evidence for EBLV infection was observed.

All manipulation with bats was performed by experts provided with the appropriate permits issued by the Ministry of Environment of the Slovak Republic (No.5376/2009-2.1/jan/2; No.5169/2012-2.2).

This work was supported by the Slovak Research and Developement Agency under the grant No.APVV-0605-12; VEGA No. 1/0960/12

Board No: 39 *Reservoirs of Zoonoses*

A STUDY OF THE EFFICACY OF PRODUCTS USED FOR WASHING DUCK EGGS

A. Rabie, B. Gosling, F. Martelli, R. Davies

Bacteriology, Ahvla, New Haw, United Kingdom

A study was conducted to investigate the efficacy of commercial products that are being used on British duck farms to wash and bleach duck eggs prior to sale. This study was initiated following outbreaks of Salmonella in the UK caused by duck eggs.
Nine products with active ingredients of potassium monopersulphate, polyhexamethylene biguaide (PHMB), sodium carbonate (NA2CO3), chlorine dioxide (ClO2), sodium hypochlorite (NaClO), peracetic acid, chloramineT and warm water as a control were evaluated using Salmonella- challenged, faecally contaminated eggs, following methods provided by GB duck egg producers. Salmonella was added to poultry faeces (1:1) at concentrations of 103, 104, 105, 106, 107 and 108cfu/g.
After 1 minute contact, ClO2 and sodium carbonate or hypochlorite based products were the only products to reduce Salmonella by 5 logs, however with a longer contact time of 7 minutes almost all of the products reduced Salmonella counts by 5 logs. The warm water wash control was positive for Salmonella throughout, except for 1 egg each at the 103 and 104 contamination levels. Total bacterial counts were only reduced after 7 minutes contact time by ClO2, peracetic acid and sodium based products.
Four eggs per product were used for the screening; later studies tested 20 eggs per product, with 1 minute contact time, at Salmonella challenge concentrations of 103, 104, 105 and 106, and although total bacterial counts were low overall, initial results suggest all products were more effective at reducing total bacterial counts than warm water alone.

Board No: 40 *Reservoirs of Zoonoses*

OCCURRENCE OF SHREW- AND MOLE-BORNE HANTAVIRUSES IN GERMANY

L. Radosa[1], M. Schlegel[2], S. Essbauer[3], D. Höper[4], B. Walther[5], D.H. Krüger[1], B. Klempa[1,6], R.G. Ulrich[2]

[1]Institute of Virology, Charité Medical School, Berlin, Germany, [2]Institute for Novel and Emerging Infectious Diseases, Friedrich Loeffler Institut, Greifswald Isle of Riems, Germany, [3]Department Virology and Rickettsiology, Bundeswehr Institute of Microbiology, München, Germany, [4]Institute for Virus Diagnostics, Friedrich Loeffler Institut, Greifswald Isle of Riems, Germany, [5]Erminea Gmb H, Erminea Gmb H, Münster, Germany, [6]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Since 2007, more than 20 new hantaviruses associated with insectivores were described worldwide. New hosts as bats, shrews and moles completely changed our view on hantavirus ecology, previously believed to be only rodent-borne viruses. In previous years, our extensive molecular screening of insectivores and subsequent sequence analyses revealed presence of two shrew-borne hantaviruses in Central Europe, Seewis virus (associated with Eurasian common shrew, *Sorex araneus*) and Asikkala virus (associated with Eurasian pygmy shrew, *Sorex minutus*), and showed the broad geographical distribution, high genetic divergence, and strong geographic clustering of both viruses. Here we report on the detection and genetic characterization of the mole-borne Nova virus (NVAV), associated with European common mole (*Talpa europaea*), from Germany.

Altogether, 21 mole samples from all over Germany were screened by genus-specific screening RT-PCR assay targeting large (L) genomic segment. Two samples from Haselschacher Buck area (Bötzingen) were found positive and additional sequences were obtained from small (S) and large segment by next-generation sequencing, as well as by sequencing of overlapping PCR fragments. Sequence comparisons with NVAV strains from Hungary and France showed a high amount of silent mutations leading to a high degree of sequence diversity on nucleotide level (86.6-87.3% sequence identity) but nearly identical amino acid sequences (98.4-98.9% sequence identity). Phylogenetic analyses confirmed that the German strains form a separate clade within the monophyletic group of NVAV sequences.

In summary, our studies showed the presence and revealed first genomic sequence data for the shrew-borne Seewis and Assikala viruses and the mole-borne NVAV in Germany. Further steps will aim for development of new diagnostic tools and evaluation of the public health relevance of these new insectivore-borne viruses.

Board No: 41 *Reservoirs of Zoonoses*

ZOONOTIC SURVEILLANCE FOR RICKETTSIAE IN DOMESTIC ANIMALS IN SOUTH-WESTERN TANZANIA

M. Starke[1], G. Dobler[1], N. Heinrich[2]

[1]Department for Virology and Rickettsiology, Bundeswehr Institute of Microbiology, Munich, Germany, [2]Department of Infectious Diseases and Tropical Medicine, University of Munich, Munich, Germany

850 ticks were collected from domestic animals from South-Western Tanzania in 2009 and stored at -80°C until now. In this study we investigated the first 172 ticks for the presence of rickettsiae. The ticks were identified using standard taxonomic keys and 16s PCR to belong to three tick genera: *Amblyomma*, *Rhipicephalus* and *Hyalomma*. Ticks of the genera *Rhipicephalus* (113/172) and*Amblyomma* (55/172) were the most abundant in domestic animals at 65,70% and 31,98%, respectively, compared to *Hyalomma* (2,33%). After identifying the tick species, DNA was extracted using the MagnaPure system (Roche, Rotkreuz, Switzerland) and the tick DNA was screened for evidence of rickettsiae using a quantitative real-time PCR assay that amplifies a 100 bp fragment of a highly conserved gene encoding the citrate synthase (*gltA*) shared by all *Rickettsia* spp. Of the 172 adult ticks collected, 104 were found to be infected with *Rickettsia*. *Rickettsia* infections were highest in *Amblyomma* (48,08%), followed by *Rhipicephalus* (49,04%), with *Hyalomma* (2,88%). All *Rickettsia*-positive tick DNA samples were then amplified and sequenced with primer sets that target rickettsial outer membrane genes (*ompA* and *ompB*), the surface cell antigen 4 (*sca*4) and the 23S rRNA gene. Of the 104 ticks that were found to be infected, 70 (72,8%) amplified at least with one gene target. After sequencing *R. africae* was found to be the dominant species and accounted for 93,10% (n=69) of the identified rickettsiea. The others were *R. massiliae* (6,9%, n=1).*Amblyomma* ticks were the main carriers of *R. africae* (50/69), whereas *R. massiliae* could be found in *Rhipicephalus evertsi*. The percent identity of the *Rickettsia* species to reference sequences ranged between 97% and 100%.
R. africae, which is the most widespread spotted-fever group rickettsia in sub-Saharan Africa and causes African tick-bite fever, which is characterized by acute, influenza-like syndromes, was present in 60,47% of the ticks collected. This huge number of infective ticks shows the high risk for African tick-bite fever in Tanzania and the importance for a raised awareness of this rickettsiosis.

Board No: 42 *Reservoirs of Zoonoses*

THE ROLE OF HARES IN THE EPIDEMIOLOGY OF *LEISHMANIA INFANTUM* IN GREECE

C.N. Tsokana[1], C. Sokos[1], A. Giannakopoulos[1], P. Birtsas[2], K. Papaspyropoulos[3], G. Valiakos[1], V. Spyrou[4], D.C. Chatzopoulos[1], M. Kantere[1], A. Touloudi[1], L.V. Athanasiou[5], A.R. Burriel[1], C. Hadjichristodoulou[6], C. Billinis[1]

[1]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece, [2]Department of Forestry and Management of Natural Environment, Technological Education Institute of Larissa, Karditsa, Greece, [3]Research Division, Hunting Federation of Macedonia &Amp;thrace, Thessaloniki, Greece, [4]Department of Animal Production, Technological Education Institute of Larissa, Larissa, Greece, [5]Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece, [6]Department of Hygiene and Epidemiology, University of Thessaly, Larissa, Greece

Leishmaniasis, caused by parasitic protozoans of the genus *Leishmania*, is a vector borne disease of public health and veterinary concern. The disease is endemic in many European countries, including Greece [1]. Although dog is the most important vertebrate host and the main reservoir of *L. infantum*, the existence of a sylvatic cycle of leishmaniasis, independent of dogs, has been suggested as a possible cause of the lack of success of control measures [2]. *L. infantum* infection has been reported in carnivores, lagomorphs, and rodents in Europe. However, the ability of a wild species to act as a potential reservoir has been demonstrated in only two species, the black rat (*Rattus rattus*) in Italy and the Iberian hare in Spain due to their capacity to infect sandflies [2,3]. Since the outbreak of leishmaniasis in Spain, lagomorphs attracted the interest of researchers, and high seroprevalences or prevalences of infection have been reported from different hare populations [3-5].

Our study aimed to evaluate the occurrence of *Leishmania* infection in hares in Thessaly, Greece a highly endemic region, where a significant increase in dog seropositivity and human leishmaniasis cases has been noted in recent years. We present the preliminary results of our ongoing study concerning the infection prevalence in hares and dogs in Thessaly in the context of the research funding program THALES. A pool of liver and spleen samples from 30 hares and lymph node aspirates and conjunctival swabs from 107 dogs suspected to be infected, were tested by PCR (ITS1 nested PCR). The sequencing performed in hare and canine positive samples, confirmed *L. infantum* infection. The environmental parameters including land uses, altitude and distance from human settlements were derived from the ArcGIS 10.1 Geographical Information Systems (GIS) software and the

ArcGIS online application (ESRI, Redlands, CA, USA). Given that under specific circumstances such as an unusually high concentration of hares, high density of sandflies and a low level of immunity in the human population, this species can act as a reservoir host, any possible association between the human leishmaniasis cases and the prevalence of infection in hares deserves further investigation.

References:

[1] Ntais P, Sifaki-Pistola D, Christodoulou V, Messaritakis I, Pratlong F, Poupalos G, et al. Leishmaniases in Greece. Am J Trop Med Hyg 2013;89:906–15.
[2] Millán J, Ferroglio E, Solano-Gallego L. Role of wildlife in the epidemiology of Leishmania infantum infection in Europe. Parasitol Res 2014;113:2005–14.
[3] Molina R, Jiménez MI, Cruz I, Iriso A, Martín-Martín I, Sevillano O, et al. The hare (Lepus granatensis) as potential sylvatic reservoir of Leishmania infantum in Spain. Vet Parasitol 2012;190:268–71.
[4] Ruiz-Fons F, Ferroglio E, Gortázar C. Leishmania infantum in free-ranging hares, Spain, 2004-2010. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2013;18:20541.
[5] Moreno I, Álvarez J, García N, de la Fuente S, Martínez I, Marino E, et al. Detection of anti-Leishmania infantum antibodies in sylvatic lagomorphs from an epidemic area of Madrid using the indirect immunofluorescence antibody test. Vet Parasitol 2014;199:264–7

Acknowledgements:

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

Board No: 43 *Reservoirs of Zoonoses*

GENETIC CHARACTERIZATION OF HEPADNAVIRUS IN SWINE FROM SLAUGHTERHOUSES IN RIO DE JANEIRO, BRAZIL

Y.R. Vieira[1], D. Santos[2], M. Portilho[1], L. Villar[1], M. Pinto[1], V. de Paula[1]

[1]Department of Virology, Oswaldo Cruz Institute, Rio De Janeiro, Brazil, [2]Department of Veterinary Microbiology and Immunology, Rural Federal University of Rio De Janeiro, Seropédica, Brazil

Background and aims: *Hepadnaviridae* family is known for Hepatitis B virus (HBV) and HBV-related viruses circulating in mammalian (humans, non-human primates - including chimpanzees, gibbons, gorillas, orangutans, woolly monkeys-, swine and bats), rodents (woodchuck and squirrels) and avian (ducks, geese, herons, storks and chickens). The inclusion of swine in this list was due to serological studies recently developed in China. No molecular data about this variant is yet available. For this reason, the aim of this study was to genetically characterize the *Hepadnavirus* in samples from domestic pig herds.
Methods: For this purpose, 36 domestic pigs from inspected slaughterhouses located in small towns from Rio de Janeiro State, Brazil were screened for Hepadnavirus-DNA. Bile and liver samples were analyzed by partial genome amplification, direct sequencing and viral load quantification.
Results: Hepadnavirus-DNA were detected in 4 swines by semi-nested PCR specific for HBV ORF S (972 bp) and ORF C (392 bp). The viral loads ranged from 0.8x103 to 1x105 copies/mL. Furthermore, phylogenetic reconstruction using partial nucleotide sequences of ORF S showed a close relationship of *Hepadnavirus* strains from swine and *Hepadnavirus* nucleotide sequences from human (from 98.9 to 99.7%), non-human primates (from 84.6 to 96.1%), rodents (from 71.1 to 72.8%) and birds (from
44.7 to 47.7 %).
Conclusions: Additional studies are needed to determine the role of swine as *Hepadnavirus* reservoirs, the potential infectivity of this agent, as well as the risk assessment.
Financial support: IOC/Capes

Board No: 44 *Reservoirs of Zoonoses*

PREVALENCE AND DIVERSITY OF *CAMPYLOBACTER* SPP. IN POULTRY SAMPLES FROM RETAIL IN THE CZECH REPUBLIC

I. Kolačková[1], R. Karpišková[2], H. Vojkovska[1], J. Bardoň[3,4], V. Pudová[4], M. Röderová[4], T. Štosová[4], M. Kolář[4]

[1]Department of Bacteriology, Veterinary Research Institute, Brno, Czech Republic, [2]Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [3]Department of Bacteriology, State Veterinary Institute, Olomouc, Czech Republic, [4]Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Poultry meat has been recognised as one of the most important source of *Campylobacter* spp., the most common bacterial pathogen causing human gastrointestinal tract infections worldwide. Two main species *C. jejuni* and *C. coli* are predominantly detected in humans. Poultry is recognised as one of the main source of these pathogens.
In this study we focused on the prevalence, species distribution and clonal subtypes of campylobacter in broiler meat originating from different suppliers. Poultry samples were purchased in retail market and tested for *Campylobacter* spp. using guideline EN ISO 10272. Species determination was performed by PCR method or MALDI-TOF MS and clonal distribution was studied by macrorestriction analysis.
The set of 107 samples was composed of 51 fresh and 56 frozen poultry. They were delivered from 7 suppliers (8 up to 22 samples from each supplier) and samples were taken within the whole year period.
Altogether almost 62% of samples were positive for the presence of *Campylobacter* spp., with higher prevalence in fresh samples (76.4%). Positive results in frozen samples were obtained in 48.2%. Samples from all suppliers (slaughterhouses) were positive for campylobacters with the prevalence rate from 44 to 100%.
C. jejuni was detected in 41 samples (62.2%), *C. coli* in 17 samples (25.8%) and 8 broilers were positive for both species.
Macrorestriction profiles showed high heterogenity in both sample types - fresh and frozen meat.

Acknowledgements:
Supported by the project IGA MZCR NT/14392 and by the MEYS of the CR under the NPU I program.

Board No: 45 *Reservoirs of Zoonoses*

CHARACTERIZATION OF TWO VIM-1 CARBAPENEMASE- INCHI2 PLASMIDS RECOVERED FROM *ESCHERICHIA COLI* AND SALMONELLA ISOLATES FROM A PIG FARM IN GERMANY

L. Falgenhauer[1,2], H. Ghosh[1,2], B. Guerra[3], Y. Yao[1,2], M. Fritzenwanker[1,2], J. Fischer[3], R. Helmuth[3], C. Imirzalioglu[1,2], T. Chakraborty[1,2]

[1]Institute of Medical Microbiology, Justus Liebig University Giessen, Giessen, Germany, [2]German Center for Infection Research (DZIF), Partner Site Giessen Marburg Langen, Justus Liebig University Giessen, Giessen, Germany, [3]Department for Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

Introduction and purpose: Resistance to carbapenems is an emerging problem in human medicine. Recently, carbapenemases have also been isolated from food animal settings. It is important to understand how the food animals acquired the resistant bacteria and if resistance genes can be transferred to humans through the food chain. As carbapenemases are often plasmid-located, a transfer of the plasmids or the strains harbouring these might be a public health concern. In this study, a comparison of two VIM-1-encoding plasmids isolated from *Escherichia coli* and *Salmonella enterica* from a same pig farm at different time points was performed, using sequencing and comparative genome analysis, to determine the source and relationships among plasmids.

Methods: Plasmids from *Escherichia coli* (pRH178) and *Salmonella enterica* (pRH27) were sequenced to determine the source and relationships of these plasmids using comparative genome analysis. Reads were assembled to contigs, and predicted gaps were closed by PCR. Comparative analysis of the two plasmids was performed using the blastn algorithm.

Results: The pRH-R178 was sequenced to closure and comprised 223,382bp while pRH-R27 was assembled to 2 contigs of around 300kb. Our results revealed that both plasmids are highly homologous and share a common backbone. Comparative genome analysis indicated that plasmid pRH-R178 is a deletion derivative of plasmid pRH-R27, with loss of 4 regions. In particular a deletion in the region comprising the origin of transfer in pRH-R178 renders this plasmid non-conjugative while plasmid pRH-R27 carries an intact transfer region and is conjugative. In addition to blaVIM-1, the plasmids harboured other antibiotic resistance genes (strA/strB conferring streptomycin resistance, blaACC-1 (conferring resistance to third generation cephalosporins) as well as resistance genes against heavy metals (copper, arsenate mercury and silver resistance).

The blaVIM-1 genes were located on an integron, together with aminoglycoside resistance genes (aacA4 and aadA1, kanamycin/gentamicin and streptomycin respectively), sulfadiazines (sul1), and ammonium quaternarium compounds (qacEΔ, detergence resistance).

Conclusions: The occurrence of carbapenem-resistant Gram-negative bacteria is rare in animal environments. As carbapenems are not used in animal settings, we propose horizontal gene transfer due to selective pressure by other antimicrobial resistance genes, detergent resistance genes and/or heavy metal resistance genes.

Board No: 46 *Risk Assessment & Public Health*

EFFECTIVENESS OF AN ALTERNATIVE APPROACH IN RABIES CONTROL ACTIVITIES IN SRI LANKA

R. Balasubramaniam[1,2], K. Kasturiratne[1], K. Jayasinghe[3], A.R. Wickeremasinghe[1]

[1]Public Health, Faculty of Medicine, University of Kelaniya, Kelaniya, Sri Lanka, [2]Rabies Diagnosis and Research, Medical Research Insititue, Colombo 8, Sri Lanka, [3]Medical Supplies Division, Ministry of Health, Colombo 10, Sri Lanka

Background: In Sri Lanka, a rabies endemic country, elimination (culling) of stray dogs which has happened since 1975 as a method to control human rabies was completely halted in 2007. This was replaced by intensified programme of sterilization and vaccination of dogs. Assessing the effectiveness of this alternative approach is crucial. Since incidence of human rabies continues to decline after 2007, this study compares two other indicators, rabies post exposure prophylaxis (PEP) and laboratory diagnosis of animal rabies before (2002-2007) and after (2008-2011) introducing this alternative approach.

Methods: Rabies PEP immunologicals supply data were extracted from the electronic records maintained at the Central Procurement Agency. Quantity and cost of rabies PEP immunologicals supplied during (2002- 2007) period was compared with that of 2008-2011. Confirmed cases of animal rabies data were extracted from the records maintained at the Central Rabies Laboratory/ Medical Research Institute. Changing trend in the number and types of animal rabies during (2002–2007) was compared with that of (2008-2011). Data were analysed using descriptive statistics such as trends and means. .

Results: In the PEP supply data, trends in key indicators namely, quantity of anti-rabies vaccine (ARV), quantity of equine rabies immunoglobulin (ERIG), cost of ARV and cost of ERIG were fluctuating during 2002-2011 with more towards declining in the latter 2-3 years. Apart from cost of ARV, annual means of other three indicators were lower during 2008-2011 than that of 2002-2007.

In the animal rabies data, trends in key indicators namely, numbers of total brains tested, total brains found positive, dog brains tested and dog brains found positive were declining in the latter 2-3 years in contrast to increasing trends seen before 2008. However, the annual means of all four indicators were higher during 2008-2011 than that of 2002-2007.

Conclusion: The alternative approach of halting stray dog elimination and replacing it with sterilization and vaccination of dogs appears to be effective in controlling human rabies in Sri Lanka. Extending study-period, expanding laboratory-surveillance, analysing multi-source data and removing the effect of confounding variables (eg: unit cost of ARV) in analysis are required to confirm this observation.

Board No: 47 *Risk Assessment and Public Health*

GENOME ANALYSIS OF FOOD-BORNE SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* FOR RISK ASSESSMENT

E. Hauser[1], T. Semmler[2], I. Eichhorn[2], L.H. Wieler[2], A. Bauwens[3], H. Karch[3], H. Schmidt[1]

[1]Department of Food Microbiology, University Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, Germany, [2]Department of Veterinary Medicine at the Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany, [3]Institute for Hygiene, University of Münster, Münster, Germany

Shiga toxin-producing *E. coli* (STEC) are among the most important food-borne pathogens causing symptoms in human from mild diarrhea to the hemolytic uremic syndrome. More than 400 serotypes are described with high diversity in isolates from contaminated food [1]. How far these strains pose a risk for humans is under investigation especially if one of the main virulence factors the "locus of enterocyte effacement (LEE)" is missing. Genes of this pathogenicity island are most often integrated in neighborhood to the tRNA genes *selC*, *pheU* and *pheV* and the gene products support amongst others the intimate binding of the bacterium to the host cell and the release of several effector proteins into the cytoplasm [2].

In this study 11 LEE-negative food-associated strains of different serotypes were under investigation by genome analysis to gain further insight in their pathogenicity potential for humans. For each strain at least one of the integration sites at *selC*, *pheU* or *pheV* was occupied analyzed by PCR [3]. The DNA of the 11 STEC strains was sequenced on an Illumina platform. Paired end reads were assembled to contigs with a mean length of about 17 kb and analyzed using Geneious software (www.geneious.com). General annotations were done by RAST (rast.nmpdr.org) and for gene comparison the BLAST algorithm was used.

First results for insertion of DNA at the *selC* locus revealed different genes and sizes of integrated DNA. One strain for example has integrated about 50 kb containing 52 open reading frames including genes for transposases, phage integrases, a channel forming transporter, a putative exoprotein involved in heme utilization or adhesion as well as DNA repair and plasmid stability. Additionally, a toxin – antitoxin system was detected. Another strain showed several phage related genes with an insertion in selC of more than 10 kb. In-depth investigation of the different gene content is necessary also in regard to antibiotic resistances to assess the pathogenicity potential of food-borne STEC strains for humans.

References:

1. Mathusa, E. C., Y. Chen, E. Enache, and L. Hontz. 2010. Non-O157 Shiga toxin-producing *Escherichia coli* in foods. J. Food Prot. 73:1721-1736.
2. Jores, J., L. Rumer, and L. H. Wieler. 2004. Impact of the locus of enterocyte effacement pathogenicity island on the evolution of pathogenic *Escherichia coli*. Int. J. Med. Microbiol. 294:103-113.
3. Hauser, E., A. Mellmann, T. Semmler, H. Stoeber, L. H. Wieler, H. Karch, N. Kuebler, A. Fruth, D. Harmsen, T. Weniger, E. Tietze, and H. Schmidt. 2013. Phylogenetic and molecular analysis of food-borne Shiga toxin-producing *Escherichia coli*. Appl. Environ. Microbiol. 79:2731-2740.

Board No: 48 *Risk Assessment and Public Health*

EXPERT ELICITATION TO ASSESS THE INFECTION RISK CATS AND DOGS POSE TO THEIR OWNERS' HEALTH

N. Möbius, K. Hille, L. Kreienbrock

Department of Biometry, Epidemiology and Information Processing, University of Veterinary Medicine Hanover, Foundation, Hanover, Germany

In more than 25% of German households live pets, among these about 8.2 million cats and 5.4 million dogs. Therefore, infectious diseases of animals may pose a risk to human health. Until now, the extent of this risk has not been exactly examined or quantified in Germany. Therefore it is the aim of this project to assess the risk for dog and cat owners to be infected.
To quantify the exposition of cat and dog owners to zoonotic pathogen a literature research on the prevalence of zoonotic pathogens in cats and dogs has been carries out. In a second step, experts from different fields (human medicine, veterinary medicine, research) were asked to rate the probability of an infection and disease of the owner if his cat or dog carries a certain pathogen. The risk for the pet owner could ranked by the experts as high, rather high, medium, rather low or negligible and was assessed for dog and cat owners as well as for children (up to 12 years) and adults (12-60 years) separately. Transmission ways of pathogens and the access of the animals to the living space of the owner were defined in advance (out-door cats and dogs with access to the bedroom).
First results will be presented to contribute to the prioritisation of the infectious risk posed by cats and dogs.

Board No: 49 *Risk Assessment and Public Health*

BETA LACTAM SUSCEPTIBILITY OF *E.COLI* CASES ISOLATED FROM DIARRHEA PATIENTS FROM SHAHREKORD TOWNSHIP

J. Kaboutari Katadj[1], S. Habibian Dehkordi[1], J. Cheraghi[2], H. Sharifi[3]

[1]Basic Sciences, Shahrekord University, Shahrekord, Islamic Republic of Iran, [2]Basic Sciences, Ilam University, Ilam, Islamic Republic of Iran, [3]Department of Food Hygiene & Public Health, Shahid Bahonar University, Kerman, Islamic Republic of Iran

Keywords: E. coli, Diarrhea, Beta lactam, Antibiotic resistance

Introduction: *E. coli* is a common causative agent of diarrhea in human and animal species which increasing antibiotic resistance made serious concern about its treatment. Because of local difference in antibiotic susceptibility, the aim of this study was to survey the Beta lactam susceptibility of *E. coli* isolated from diarrheal patients in Shahrekord Township.

Material & Methods: 234 diarrheal samples were taken from Shahrekord hospitals and by complementary bacteriological tests and PCR, 131 *E.coli* positive cases were isolated. Disk diffusion antibiogram test was done by using Penicillin V, Cefixime, Cefotaxime, Imipenem and Cephalexin.

Results: According to the standard zone of inhibition for each antibiotic used the antibiogram pattern is shown in the below table.

Antibiotic	Resistance	Susceptible
Penicillin V	94.7%	5.3%
Cefixime	77.9%	22.1%
Cefotaxime	80.9%	19.1%
Imepenem	35.1%	64.9%
Cepahlexin	83.2%	16.8%

Conclusion: Imipenem with the highest antibiotic susceptibility seems to be a suitable choice in the treatment of *E. coli* diarrhea while Penicillin V with 94.7% of resistance seems to be ineffective in this regard. Thus serious attention in selecting suitable antibiotic is necessary for successful treatment and decreasing the risk of more spread of antibiotic resistance.

Board No: 50 *Risk Assessment and Public Health*

FATAL INFECTIONS CAUSED BY METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* OF CLONAL COMPLEX CC398: CASE PRESENTATIONS AND MOLECULAR EPIDEMIOLOGY

C. Berning[1], C. Lanckohr[2], K. Becker[1], G. Peters[1], B. Kahl[1], R. Koeck[3]

[1]University Hospital Muenster, Institute of Medical Microbiology, Muenster, Germany, [2]University Hospital Muenster, Department of Anesthesiology, Intensive Care, and Pain Medicine, Muenster, Germany, [3]University Hospital Muenster, Institute of Hygiene, Muenster, Germany

Methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 as determined by multilocus sequence typing (MLST) have emerged in livestock. Many studies have also described cases of human infections due to MRSA CC398 including endocarditis or pneumonia.
Here, we report on the current epidemiology of MRSA CC398 among patients at the University Hospital of Münster (UKM), located in an area characterized by a high density of pig production. In addition, we elaborate on cases of fatal infections caused by MRSA CC398 among these patients.
Methods: Every inpatient was screened for MRSA at admission using a nasopharyngeal swab. Routinely, every first MRSA isolate of each patient-case (both isolates from screenings and from clinical specimens) and additionally every MRSA isolate from blood cultures, were *spa* sequence-typed. *Spa* types were clustered in spa clonal complexes (*spa*-CC) using the Based Upon Repeat Pattern (BURP) algorithm. Additional data for lethal infections were assessed from patients' records.
Results: In 2013, 534 individual cases of MRSA were detected (including cases of both colonization and infection) among UKM inpatients. Of these, 170 (31.8%) were caused by closely related *spa*types (t034 n=79; t011 n=72; t898 n=4; t108, t1451, t2011 each n=3; t2576 n=2; t1255, t2346, t4208, t4652 each n=1) clustering in one *spa*-CC indicative for MLST CC398. At the UKM, the proportion of MRSA on all S. aureus from blood cultures was 12/97 (12.4%) in 2013 (duplicate isolates of the same patients excluded). Two of 12 MRSA isolates from blood cultures (16.7%) were characterized by *spa*-types (t034 n=1; t2576 n=1) associated with MRSA CC398. Two lethal infections due to MRSA CC398 were observed including one case of pneumonia (t011) and one case of arthritis and endocarditis (t2576). Both infections occurred in immunocompromised patients (due to lung transplantation and rheumatic disease) who had (indirect) contact with livestock animals.

Conclusion: We documented a further increase of MRSA CC398 among inpatients in Northwestern Germany. MRSA CC398 was the most frequent MRSA clonal lineage isolated in 2013. The occurrence of fatal infections highlights the importance of preventing spread of MRSA CC398 to patient groups prone to acquire severe nosocomial infections.

Board No: 51 *Risk Assessment and Public Health*

RATE OF SECONDARY TRANSMISSION OF MERS-CORONAVIRUS IN HOUSEHOLD CONTACT CLUSTERS

M.A. Mueller[1], C. Drosten[1], B. Meyer[1], V.M. Corman[1], M. Al-Masri[2], R. Hossain[3], H. Madani[2], A. Sieberg[1], B.J. Bosch[4], E. Lattwein[5], R.F. Alhakeem[2], A. Assiri[2], W. Hajomar[6], A. Albarrak[7], J.A. Al-Tawfiq[8,9], A. Zumla[2,10], Z.A. Memish[2,11]

[1]Institute of Virology, University of Bonn Medical Centre, Bonn, Germany, [2]Global Centre for Mass Gatherings Medicine, Ministry of Health, Riyadh, Saudi Arabia, [3]Regional Laboratory, Ministry of Health, Jeddah, Saudi Arabia, [4]Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, Netherlands, [5]R&D Serology, Euroimmun Ag, Lübeck, Germany, [6]Regional Laboratory, Ministry of Health, Riyadh, Saudi Arabia, [7]Devision, Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [8]Saudi Aramco Medical Services Organization, Saudi Aramco, Dhahran, Saudi Arabia, [9]Devision, Indiana University School of Medicine, Indianapolis, United States, [10]Division of Infection and Immunity, Ucl Hospitals, London, United Kingdom, [11]Devision, Alfaisal University, Riyadh, Saudi Arabia

Middle East respiratory syndrome coronavirus (MERS-CoV) causes an ongoing epidemic on the Arabian Peninsula. Strategies to contain MERS-CoV depend on knowledge of the rate of human-to-human transmission including subclinical infections. A lack of validated diagnostic tools has hindered targeted transmission studies.

We studied 26 different MERS-CoV index cases and their 280 close household contacts who had no or subclinical respiratory symptoms. Molecular evidence for MERS-CoV transmission was obtained by two independent RT-PCR assays performed on respiratory tract samples. Serological evidence was given by a comprehensive serological investigation using a sensitive and specific enzyme-linked immunosorbent assay (ELISA) followed by a staged confirmatory diagnostic based on two different immunofluorescence assays (IFA) and virus neutralization test (NT). Data were complemented by IgM testing as well as specific differential serology for all known human CoV which cause mild respiratory tract disease and induce cross-reacting antibodies against MERS-CoV.

The molecular and serological investigation suggested that 9/280 subjects (3.2%) were infected by 26 index cases (34.6%, 95% CI, 19.4-53.8%). In 7/280 contacts (2.5%) MERS-CoV nucleic acids could be detected in respiratory samples. Serological evidence could be obtained in 2/280 contacts (0.7%) by specific detection of anti-MERS-CoV antibodies in all applied assays including a highly specific virus neutralization test (NT titers >1:40). These data may enable a validation and re-adjustment of estimates of fundamental epidemiological parameters for MERS-CoV in humans.

Board No: 52 *Risk Assessment & Public Health*

OCCURRENCE OF *TOXOCARA CANIS* EGGS IN DOG POPULATION AND THEIR PRESENCE IN ENVIRONMENT

I. Papajova, J. Pipikova

Department of Environmental and Plant Parasitology, Institute of Parasitology SAS, Kosice, Slovakia

Regarding the spread of parasitozoonoses, domestic animals (dogs, cats) are of great importance because they live in a close contact with man. A new issue has arisen as a result of the growth of the pet population in cities as well as in the countryside - and it is animal waste treatment, as faeces are potential source of infective stages of endoparasites. The aims of this study were as follows: to monitor the occurrence of the propagative stages of intestinal endoparasites in dogs excrements from selected towns in Slovakia and to study parasitological contamination of sandpits in this towns. Totally 211 dog's faecal samples from 4 towns of Slovakia were examined for the presence of helminth eggs with 25.6 % of the samples being positive. The most prevalent in faeces were the eggs of *Toxocara canis*. *Toxocara* spp. eggs were found in 5.7 % sandpits from Košice, 10.0 % from Prešov, 16.7 % from Trenčín and 30.0 % from sandpits in Zvolen. Environment contaminated with eggs of endoparasites might pose the potential risk for spread of parasitosis among animals, but also in human population.

This study has been realized thanks to the financial support of the project VEGA no. 2/0140/13.

Board No: 53 *Risk Assessment and Public Health*

DEVELOPMENT OF SURVEILLANCE SYSTEM FOR EARLY DIAGNOSIS OF TICK-BORNE ZOONOSES IN PIEDMONT, ITALY

M.D. Pintore[1], L. Tomassone[2], L. Ceballos[2], A. Pautasso[1], F. Giorda[1], M. Bardelli[3], F. Rizzo[1], M.L. Mandola[1], S. Peletto[1], A. Torina[4], C. Casalone[1], A. Mannelli[2], B. Iulini[1]

[1]Istituto Zooprofilattico Sperimentale Del Piemonte, Liguria E Valle D'aosta, Turin, Italy, [2]Department of Veterinary Sciences, University of Turin, Turin, Italy, [3]Asl, Vco, Verbania, Italy, [4]Istituto Zooprofilattico, Sperimentale Della Sicilia, Sicilia, Italy

Keywords: surveillance, ticks, zoonoses.

Ticks can transmit infections involving both humans and animals. Tick-Borne Zoonoses (TBZ) have increased in Europe over the last decade and human risk is linked to the density of questing ticks, in particular to the nymphs density. Twelve clinical cases of borreliosis were officially diagnosed since 2008 in Verbano-Cusio-Ossola (VCO) Province, northeastern Piedmont in Italy. The aims of the study were to evaluate the tick abundance in VCO province and regional Parks of Mandria and Stupinigi (Turin province) and to investigate the prevalence of zoonotic pathogens in questing ticks and ticks from bitten patients.
Sites located at different altitudes were selected and questing ticks were monthly collected by dragging from April to September 2011-2012. Ticks from bitten patients were also investigated.
A total of 4434 ticks were collected (3192 in VCO province, 908 in the Regional Parks and 334 from man) and identified. A statistically significative sample was selected for molecular analyses. In VCO all ticks were identified as *I. ricinus*, and most of them were found at an altitude between 1000 and 1200 m above sea level. Three genera of ticks were collected in the regional Parks (75.6% *Ixodes*, 24% *Haemaphysalis* and 0.4% Dermacentor) and from humans (78.8% Ixodes, 0.9% Rhipicephalus and 0.3% *Dermacentor*). Preliminary tests targeting *Borrelia burgdorferi sl*, *Rickettsia* spp., *Anaplasma* spp. in questing ticks show an infection prevalence of 10.4%, 4.3% and 1.5% respectively, and in ticks from humans of 4.5%, 16% and 3.4% respectively. No Tick Borne Encephalitis virus (TBEV) was found. Borrelia positive samples were sequenced, and four genospecies were found: *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. lusitaniae*. Finally, phylogenetic analysis based on the OspC gene showed that most of the Borrelia strains from pathogenic genospecies might have the potential for human infection and for secondary invasion.

TBZ in humans are correlated with local tick abundance, density of vertebrate reservoir hosts, climate changes and ticks infection prevalence. Analyses of these factors can help in assessing the risk and guide the implementation of public health policies against TBZ.

Board No: 54 *Risk Assessment and Public Health*

DOMESTIC ANIMALS AS A SOURCE OF PARASITIC ZOONOSES IN THE LOCALITIES WITH LOW HYGIENIC STANDARDS

J. Pipikova, I. Papajova

Department of Environmental and Plant Parasitology, Institute of Parasitology SAS, Kosice, Slovakia

Parasitic zoonoses are diseases typical for developing countries and for socio-economically disadvantaged areas, which are often characterized by low hygiene standards and coexistence of large number of people and animals in a small space. Domestic animals such as dogs and cats are important source of parasites, which can be dangerous for human health and the transmission of parasitic germs from animals to humans happens often through contaminated environment. In Slovak Republic such type of environment is represented mostly by villages with segregated Roma settlements.

The aim of this study was to observe the occurrence of parasitic zoonoses in dogs' population in the localities with low hygienic standards, and also to detect the presence of parasitic germs with zoonotic potential in soil and occurrence of parasitic diseases in children population in model localities – Rudňany and Petrová.

Total 109 samples of dogs' faeces, 51 samples of stool and 10 samples of soil were collected in model localities. 90.32% of dogs' excrements from Rudňany and 97.87% of samples from Petrová contain at least one species of endoparasite. 11 different species of intestinal parasites were detected in faecal samples from selected localities as follows: eggs of family Ancylostomatidae (83.49%),*Ascaris* spp. (55.96%), *Toxocara* spp. (41.28%), *Trichuris* spp. (24.77%), oocysts of *Isospora* spp. (21.10%), eggs of *Toxascaris leonina* (18.35%), sporocysts of *Sarcocystis* spp. (16.51%), cysts of *Giardia duodenalis* (11.92), eggs of *Capillaria* spp. (5.50%), eggs of family Taeniidae (5.50%) and *Dipylidium caninum* (0,92%). In 10 samples of soil the eggs of *Ascaris* spp. (6 samples), *Toxocara* spp. (2 samples), family Ancylostomatidae (1 sample), *Capillaria* spp. (1 sample) and eggs of family Taeniidae (1 sample) were detected. In stool samples from children population the eggs of *Ascaris lumbricoides* (58.82), *Trichuris trichiura* (11.76%), *Hymenolepis nana* (3.9%) and cysts of *Giardia duodenalis* (1.9%) were detected.

Based on the results, we can conclude that the main sources of parasitic zoonoses in the studied localities are domestic animals. Increased risk of transmission of parasitozoonoses is predominantly in the areas with low hygienic standards, low level of health awareness, and poor level of technical infrastructure.

This study was supported by project VEGA no. 2/0140/13.

Board No: 55 *Risk Assessment and Public Health*

PREVALENCE OF CLOSTRIDIUM DIFFICILE AND DIVERSITY OF GENOTYPES COLONIZING COMPANION ANIMALS AND THEIR OWNERS: A GERMANY-WIDE SURVEY

D. Rabold[1], W. Espelage[2], F. Grzebin[2], T. Eckmanns[2], N. Pfaffendorf-Regler[3], A. Schneeberg[3], L.H. Wieler[4], A. Lübke-Becker[1], C. Seyboldt[3]

[1]Ag Infektionsdiagnostik Und Molekulare Epidemiologie, Institut Für Mikrobiologie Und Tierseuchen, Freie Universität Berlin, Berlin, Germany, [2]Abteilung Für Infektionsepidemiologie, Robert Koch Institut, Berlin, Germany, [3]Institut Für Bakterielle Infektionen Und Zoonosen, Friedrich Loeffler Institut, Bundesforschungsinstitut Für Tiergesundheit, Jena, Germany, [4]Institut Für Mikrobiologie Und Tierseuchen, Freie Universität Berlin, Berlin, Germany

Clostridium (*C.*) *difficile* infection (CDI) can vary from symptomless carriage to life-threatening disease of the intestine in humans. Recent changes in epidemiology of CDI with increasing incidence and severity are of particular concern. Virulent strains affecting humans have also been isolated from various animal species. However, scarce epidemiological data on *C. difficile* in companion animals limits the knowledge about possible interspecies transmission. This study aimed to collect first national data on occurrence and genotypic variation of *C. difficile* in dogs, cats and their owners.

A Germany-wide survey sampling companion animals and their owners was conducted from July 2012 to August 2013. In 415 different households 1,435 participants contributed faecal samples with 59.3% being of animal and 40.7% human origin. Capillary gel electrophoresis based PCR ribotyping, Multilocus VNTR Analysis (MLVA) and PCR detection of toxin genes A, B and the binary toxin were used to characterise isolated C. difficile strains.

The *C. difficile* isolation rates were 2.94% (25/851) and 2.91% (17/584) for animal resp. human samples. Typing revealed eight different PCR ribotypes in isolates from companion animals. Three of those were also isolated from human samples (014/0, 010 and 078). Within two households identical ribotypes were isolated from two partner animals (in both cases 014/0), whereas no *C. difficile* pair from owner and pet sharing the same household could be detected.

Board No: 56 *Risk Assessment and Public Health*

EMERGING TRICHINELLA INFECTIONS IN PIGS RAISED UNDER UNCONTROLLED CONDITION IN POLAND

M. Rozycki, E. Bilska-Zajac, E. Chmurzynska, T. Cencek

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pulawy, Poland

Examination of pigs against Trichinella, in Poland has long established practice reefer back to the end of XIX century. In Greater Poland examinations of pigs were obligatory since 1879y. Formerly examinations were provided by trichinoscopic method, since late 70 - ties of the XX century the digestive method was introduced. Nowadays examinations of meat are compulsory even if the slaughter takes place at farm and the pork is for owner's purpose only, examinations are done according to Annex I Commission Regulation (EC) No 2075/2005.
The number of human infections during the last decade varies from 6 to 297 with an average of 80 and median 46. By 1995 most of the infections were caused by ingestion of infected pig meat. Nowadays most of infection has source in consumption of wild boar meat. Official data on trichinella are present as the sum of infected animals per province per year. This data gives general approach to the problem but their usefulness in epidemiology is limited. Up to 2013 the only sporadic pig infections were documented, and year by year epidemiological situation improved. In 2010 for the control of pig trichinellosis, immunoserological monitoring was launched. Each year over 1600 pig sera were tested, with negative results. In 2013 in 3 herds (backyard raised) outbreaks of trichinella occurred. In suspected herds targeted monitoring was launched. All pigs from those herds were considered as suspected and undergo official restriction. Seropositive pigs were slaughtered and the rest of pigs were retested 36 days later. In the first focus one pig (saw) was found seropositive, one month later no additional pigs were found seropositive. In the next focus 49 samples of blood were taken, 14 pigs were found to be positive. Infected pigs were slaughter and the meat was condemned. Meat samples were digested, burden of larvae varied from 0,3 to 4,9. One month later all pigs from the suspected herd were slaughtered. Samples of meat and blood were tested. In fattening pigs (30-120kg) 31 animals were found positive by digestive method, in the same group only 29 serum samples were seropositive (93,5%). Over 6% of trichinella positive pigs were false negative in ELISA.

In the next suspected herd, 36 pigs were examined of which 6 were seropositive (four saw and 2 fattenig), two month later new 17 pigs were found seropositive. As confirmatory test for ELISA, Western blott test was used. Western blot analysis confirmed ELISA results in 15 cases. In 2 cases in Western blotting unspecific pattern was obtain. Obtained results confirm the gaps in ELISA test and necessity of confirmation of ELISA results by Western blot. The etiological agent was identified as Trichinella spiralis. The average larval burden was law as 1,6 lpg in the masseter muscle. In all cases production methods were close to organic farming. Exposure of animals to Trichinella and other parasitic agents was possible through rodents or other vectors. The low cost production has grown in popularity over the last 3 years due to the high cost of industrial feeds. This type of pig production increases the risk for *Trichinella* spp. infections, since animals can acquire the infection by feeding on carcasses or the offal of hunted or dead wild animals. The awareness and education of farmers is extremely important to reduce the transmission among free ranging pigs and the risk for humans.

Board No: 57 *Risk Assessment and Public Health*

PATHOGENIC NEMATODES FOUND IN FISH AND FISHERY PRODUCTS MADE OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) FROM MARKETS IN POLAND

M. Rozycki, E. Bilska-Zajac, E. Chmurzynska, T. Cencek, M. Kochanowski

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pulawy, Poland

The aim of the study was to determine the frequency and intensity of roundworm infections in highly valued fish and fishery product (mainly fillets) made from the muscle tissue of pink salmon present on the market and species identification of parasites detected. Pink salmon were caught by a commercial seiner in FAO 67 region. For the explanation of the area 67, are marine waters of the Northeast Pacific from a the mainland coast of Russia in the Western Bering Sea at 175°00'W, along the coast to Mys Dazhneva, hence through the Bering Strait to Cape Prince of Wales and in southeast direction to the mainland coast of Alaska to the 130°00'W longitude and to 40°00'N latitude. For the study over 150 samples were taken. Samples were collected from 2009 to 2012. All samples were taken at frozen stage. The average weight of the sample was 250g. Boneless, skinless fillets were placed in polyethylene bags and deiced overnight. Priori to examination fillets were gently grind by hand. Parasites were isolated by artificial digestion method. Digestion method (ZP/PB-45) was validated and accredited by Polish Centre for Accreditation (PCA). Found nematodes were identified on the base of morphological characteristics according to Grabda key identification of marine parasite entities guide. Species confirmation was performed by PCR/RFLP (EURLP developed and validated method). Parasites were found in 93 (62 %) samples. Larvae were identified on the base of morphological characteristic as *Anisakis spp.* and *Pseudoterranova decipiens*. No other parasites were found in the examined samples. Species identification were confirmed by PCR/RFLP method. Over 96 % of nematodes were identified as *Anisakis simplex sensu stricto* and 4 % as Pseudoterranova decipiens. Obtained results confirm the prevalence of human pathogenic and allergenic nematodes in highly valued fish products. Presented work is the part of studies on set of methods for detection of nematodes in fish and fish products of particular importance due to their pathogenicity and allergenicity for humans.

Board No: 58 *Risk Assessment & Public Health*

SIGNIFICANCE OF CLOSTRIDIUM BOTULINUM IN CHRONIC DISEASE OF DAIRY CATTLE

C. Seyboldt[1], S. Dischер[1], E. Jordan[1], H. Neubauer[1], M. Hoedemaker[2]

[1]Institut Für Bakterielle Infektionen Und Zoonosen, Friedrich Loeffler Institut, Jena, Germany, [2]Klinik Für Rinder, Stiftung Tierärztliche Hochschule Hannover, Hannover, Germany

For more than a decade the cause for gradual health deterioration of cattle herds has been discussed controversially. The crucial point of one of the hypotheses on the development of this clinical picture is the production of neurotoxin by *Clostridium botulinum* in the intestine of the affected animals. This hypothesis is known as visceral or chronic botulism. The University of Veterinary Medicine Hannover (TiHo) and the Friedrich-Loeffler-Institut (FLI) cooperated in a research network to investigate the cause of this complex of syndromes in dairy cows which is associated with wasting and can affect entire dairy herds. 139 farms and 10 fecal samples from each farm were investigated for the presence of *Clostridium botulinum* neurotoxin. Task of the FLI within the project was the sensitive detection of this neurotoxin in collected faecal samples. Selection and classification of the sampled animals into an epidemiological category was task of the TiHo being also the coordinator of the project. The diagnostic procedure and the results concerning the detection of *Clostridium botulinum* neurotoxin will be presented.

Board No: 59 *(New and Re-) Emerging Zoonotic Diseases*

BABESIA DETECTION IN THE BAIKAL REGION OF RUSSIA

O. Fedulina[1], V. Rar[2], O. Suntsova[1], I. Kozlova[1], O. Lisak[1]

[1]Laboratory of Molecular Epidemiology and Genetic Diagnostics, FSBI "Scientific Center for Family Health and Human Reproduction Problems" SB RAMS, Irkutsk, Russian Federation, [2]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russian Federation

Babesiosis foci are widespread in the United States, Europe and Asia. *Babesia* spp. were recently found in Russia in Western Siberia and Far East in ticks and small mammals. However, there is still no information on the presence of babesiosis foci in the Baikal region, Russia.

Totally, 625 *Ixodes persulcatus*, 158 *Haemaphysalis concinna*, and 94 *Dermacentor nuttalli* ticks, as well as 136 equine blood samples and 55 liver samples of small mammals were examined on the presence of *Babesiidae* DNA.

Babesia DNA was detected in 12 *I. persulcatus* and 5 *H. concinna* ticks from three southern districts of the Irkutsk region. The analysis of 18S rRNA gene sequences has shown that the detected *Babesia* spp. belong to both *B. microti* and *Babesia* sensu stricto clusters. Four *I. persulcatus* ticks carried the *B. microti* 'US'-type genetic variant corresponding to *B. microti* strain G1. Three*I. persulcatus* carried *B. venatorum* sequence variant which was identical to isolates previously detected in *I. persulcatus* in Russia and China but differed from the European isolates. The remaining five *H. concinna* and *five I. persulcatus* positive ticks carried six different *Babesia* s.s. sequence variants that have not been previously detected in Russia. One *H. concinna* tick carried a sequence variant closely related (similarity of 98.6%) to the *B. motasi* from a sheep in the Netherlands. One *I. persulcatus* tick carried a sequence variant belonging to a large phylogenetic group formed by *B. divergens*, *B. venatorum*, etc., which considerably differed from the known *Babesia* spp. sequences (identity of <94.7%). Other sequence variants were closely related (a similarity of 97.1–97.8%) to *B. crassa* recovered from a sheep in Iran.

In addition, *Babesiidae* DNA was detected in 47 (34.6%) of examined equine blood samples and in four (7.3%) *Microtus oeconomus* liver samples.

The study was supported by RFBR grant 14-04-32375.

Board No: 60 *(New and Re-) Emerging Zoonotic Diseases*

LEISHMANIA SIAMENSIS AS THE CAUSE OF AUTOCHTHONOUS CUTANEOUS LEISHMANIASIS OF HORSES IN GERMANY - A NEW EMERGING ZOONOTIC DISEASE?

K. Kuhls[1], D. Sinning[1], F. Rauhut[2], K. Köhler[3], L. Litzke[4], M. Frohme[1], G. Schönian[2]

[1]Molecular Biotechnology and Functional Genomics, Technische Hochschule Wildau, Wildau, Germany, [2]Institute of Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany, [3]Institute of Veterinary Pathology, Justus Liebig University Giessen, Giessen, Germany, [4]Clinic for Horses (Surgery), Justus Liebig University Giessen, Giessen, Germany

In Germany, leishmaniasis is usually considered as being imported through travelling to or from endemic regions but rare putative autochtonous human cases and cases in different animal species are also described. In 2004, a granulomateous inflammation was found at the eye lid of a horse. The animal was born in Germany and kept in Bavaria and Hesse. After surgery resected tissue was examined histopathologically and a cutaneous leishmaniasis was diagnosed. The parasites have been identified as belonging to the genus *Leishmania*, but no homology was found to any of the known species at that time. Analysis of the DNA polymerase α revealed an identical sequence to those of two strains from patients with skin lesions from Martinique. All three strains were found to be only distantly related to all other human-pathogenic *Leishmania* species.
In 2008, a new *Leishmania* species referred to as *L. siamensis* has been described as the causative agent of visceral leishmaniasis in Thailand and more sporadic autochthonous human infections were reported from there since then. Cutaneous leishmaniasis was reported in horses and a cow from Switzerland and Germany in 2009, and sequencing of the ITS-1 rDNA revealed identity to *L. siamensis*. Another case of such infection of a horse was found 2011 in Florida, USA. By comparing the ITS-1 sequence of our equine sample isolated in 2004 with these new data we could identify it as *L. siamensis*. Moreover, our data support the close phylogenetic relationship of *L. siamensis* to the zoonotic species *L. enrietti*. The occurrence of *L. siamensis* in Central Europe raises many questions, e.g. whether this is a new emerging zoonotic disease, about the origin of this parasite, the mode of transmission, and the involved vector. Further investigations are necessary to establish whether these cases found on different continents point to a new global emerging zoonotic disease or whether it has been overlooked so far because of the sporadic occurrence of such cases. This includes a detailed study of possible cases, the putative transmission cycles and the involved reservoirs and vectors in all effected countries. This knowledge will be essential for respective surveillance and control measures.

Board No: 61 *(New and Re-) Emerging Zoonotic Diseases*

OCCURRENCE OF DISINFECTANT RESISTANCE AT ESBL- / AMPC-PRODUCING *E. COLI* ISOLATES FROM CHICKEN

T. LI, N. Roschanski, H. Laube, A. Friese, U. Rösler

Department of Veterinary Medicine at the Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany

Disinfectants are widely used in different areas (e.g. medical environments, food industry, livestock associated environments etc.) but the increasing use might impose a selective pressure for emergence of disinfectant-resistances. On the other hand, the co-selection of disinfectant- and antibiotic-resistances have been reported on a range of various bacteria too (Wong et al. 2012; Coelho et al. 2013). This suggests that the use of disinfectants might be able to contribute to the spread of antibiotic resistances and pose a human health thread.
To address this topic 90 ESBL-/ AmpC-producing *E. coli* strains, isolated from cloacal swabs of chicken just before slaughtering were used to determine the minimal inhibitory concentration (MIC) against the disinfectants benzalkonium chloride (BKC), chlorhexidine (CHX), and triclosan (TCL).
Compared to the measured MIC-values of three *E. coli* reference strains (DSM682, DSM1103 and the laboratory K12-strain DH5α) 23 of the tested samples showed decreased susceptibility to single or multiple disinfectants (8 /CHX; 7 /TCL; 3 /BKC+ CHX; 5CHX+TCL). However, none of the strains was able to survive at concentrations recommended for disinfection with commercially available products or applied to sanitary products.
In addition, the distribution of four different qac genes (qacEΔ1, qacE, qacH, and qacF), which seems to be related to bacterial resistance against quaternary ammonium compounds (QACs) (Gillings et al. 2009), were investigated. The qacEΔ1 gene was found in (17/90) 18.89% samples, while the frequency of qacH (6/90) 6.67% was low and qacE, and qacF were not detected. However, all the isolates with an increased MIC against BKC have none of the investigated qac genes, while three BCK sensitive isolates contain the qacH as well as the qacEΔ1 gene. The obtained results demonstrate that *E. coli* strains possessing decreased susceptibility to disinfectants could be found within livestock but however, there is no strong correlation between the presence of qac genes and the observed phenotype. This observation indicates that there must be other mechanisms of the decreasing disinfectant susceptibility in *E.coli*. Additionally, this ongoing investigation shows that there is no obvious association between resistance against QAC disinfectants and resistance against cephalosporines at poultry.

Board No: 62 *(New and Re-) Emerging Zoonotic Diseases*

LONG-TERM SEROSURVEILLANCE OF WEST NILE AND USUTU VIRUSES IN FERAL HORSES IN GUADALQUIVIR MARSHES, DOÑANA, SPAIN

F. Llorente[1], S. Ruiz[2], M. del Carmen Barbero[1], J. Figuerola[3], J. Martínez-de la Puente[3], M. Ángel Jiménez Clavero[1], R. Soriguer[4]

[1]Centro De Investigación en Sanidad Animal, Inia, Valdeolmos, Spain, [2]Servicio De Control De Mosquitos, Diputación De Huelva, Huelva, Spain, [3]Departamento De Ecología De Humedales, Estación Biológica De Doñana (Ebd Csic), Seville, Spain, [4]Departamento De Etología Y Conservación De La Biodiversidad, Estación Biológica De Doñana (Ebd Csic), Seville, Spain

In Europe, wetland areas with large populations of birds and mosquitoes provide an optimal habitat for the emergence of arthropod-borne flaviviruses such as West Nile virus (WNV) and Usutu virus (USUV). Our previous studies in Doñana National Park (Guadalquivir marshes, Southern Spain) found seroconversions for WNV in common coots in 2004 and 8% seroprevalence in free ranging horses in 2005. This horse population is considered to be feral, since it lives free outdoors in the marshes all year round, and far away from anthropogenic habitats, so it can be useful for WNV surveillance in this natural area. Hence, in 2005 we initiated a serosurveillance study in this population of feral horses, which has been continued until 2013. The results showed a decline in WNV seroprevalence the first years (2005-08) to reach undetectable levels in horses in 2008, followed by a new period of intense WNV circulation between 2009 and 2013. In this whole period the population studied has remained asymptomatic but, remarkably, in 2010, WNV disease affected domestic horses and humans in neighboring areas, with sporadic cases since then. Interestingly, USUV virus positive sera have been detected in 2012 and 2013, showing the first evidence of circulation of these viruses in horses in Spain, as has been documented in recent years in Croatia and Italy. First evidence of USUV presence in the study area occurred in 2009, in *Culex perexiguus* mosquitoes, but positive serology in horses has not been detected until three years latter. These data indicate that two different arthropod-borne flaviviruses (WNV and USUV) are affecting horses in the Guadalquivir marshes at least since 2012, just after the detection of co-circulation of these viruses in game birds in Southern Spain.

Board No: 63 *(New and Re-) Emerging Zoonotic Diseases*

IN VITRO INHIBITION OF MERS-COV WITH ALFERON® (INTERFERON ALFA-N3)

C.A. Martellaro, H. Feldmann, D. Falzarano

DMT, RML/NIAID/NIH, Hamilton, United States

Middle East respiratory syndrome- coronavirus (MERS-CoV) causes severe respiratory illness in humans with a case-fatality rate between 30-40%. It was first reported in 2012 in Saudi Arabia and to date there are 20 countries with lab-confirmed cases. Currently there are no specific treatments recommended for illnesses caused by MERS-CoV. We have shown that Alferon®, the only commercially available natural source of alpha interferon in the US, significantly reduces replication of MERS-CoV/EMC/ 2012 in LLC-MK2 cells. At 1 day post-infection, there was more than a 1 log reduction in virus titer when cells were treated with 100 IU/ml either 18 hours prior to infection (pre-treatment) or 1 hour following infection (post-treatment). Additionally at Day 1, those cells pre-treated with 6400 IU/ml showed more than a 3 log reduction in virus titer but showed no dose dependency in those post-treated. By 3 days post-infection, both the pre- and post-treated cells showed nearly a 2 log reduction in viral replication when treated with 100 IU/ml which extended in a dose-dependent manner to more than a 4 log reduction at 6400 IU/ml. Given this data, additional testing of Alferon® in animal models is warranted to assess its potential as a candidate for the treatment of MERS-CoV infection.

Board No: 64 *(New and Re-) Emerging Zoonotic Diseases*

THE EFFECT OF *STREPTOCOCCUS SUIS* CO-INFECTION ON THE INFECTION OF WELL-DIFFERENTIATED PORCINE RESPIRATORY EPITHELIAL CELLS BY SWINE INFLUENZA VIRUSES

F. Meng[1], N. Wu[1], M. Seitz[2], P. Valentin-Weigand[2], X. Ren[3], G. Herrler[1]

[1]University of Veterinary Medicine, Hannover, Institute of Virology, Hannover, Germany, [2]University of Veterinary Medicine, Hannover, Institute of Microbiology, Hannover, Germany, [3]Northeast Agricultural University, College of Veterinary Medicine, Harbin, China

Disease often occurs due to a combination of various factors including viral and bacterial pathogens as well as environmental factors. A major factor responsible for severe virus infections may be bacterial co-infections. As known, pigs are important hosts for influenza A viruses and may play an important role in the interspecies transmission of influenza viruses. Primary target cells for Influenza viruses are the epithelial cells in the respiratory tract. Differentiated airway epithelial cells contain special cell types such as ciliated cells or mucus-producing cells that can't be maintained as immortalized cell cultures. We have recently reported a culture system for differentiated respiratory epithelial cells to analyze the infection of porcine influenza viruses in their natural target cells. Therefore, the aims of this study are to analyze the effect of *Streptococcus suis* (*S.suis*) co-infection on the infection of well-differentiated porcine respiratory epithelial cells by porcine influenza virus types H1N1 and H3N2.The comparison of mono- and co-infection reveals to what extent the bacterial infection enhances the severity of infection by porcine influenza virus. We compared five porcine viruses of the three subtypes currently prevalent in the swine populations (H3N2, H1N1, H1N2) with respect to the following parameters: (1) duration of the growth cycle; (2) amount of infectious virus released into the supernatant; (3) extent of the ciliostatic effect. These viruses showed differences in their growth behavior and ciliostatic effect on PCLS and thus reflected the virulence properties of these viruses. Our co-infection studies will reveal whether *S.suis* differentially affects influenza viruses differing in their virulence.

Board No: 65 *(New and Re-) Emerging Zoonotic Diseases*

INCIDENCE AND BIOCHEMICAL CHARACTERISTICS OF *LISTERIA MONOCYTOGENES* OF RAW GOAT MILK PRODUCED IN SHAHREKOED, IRAN

H. Moshtaghi

Food Hygiene and Quality Control, Shahrekord University, Shahrekord, Islamic Republic of Iran

Keywords: *Listeria monocytogenes*, goat raw milk, biochemical charactristics

Objectives: The role and importance of *Listeria monocytogenes* as an agent of foodborne disease is becoming increasingly appearent. Recent two decates outbreaks of listeriosis have led to the implication of a variety of foods particularly dairy products, as source of pathogen. The aim of this research was to study the incidence and biochemical characteristics of *L. monocytogenes* isolated from raw goat milk produced in Shahrekord City of Iran.

Methods: about 350 raw goat milk samples analyzed by two enrichment method. Listeria enrichment broth (LEB) and Fraser secondary enrichment broth were used as primary and secondary enrichments and streaked on PALCAM agar. The suspected isolates after purification, Gram´s staining, motility test at 20-25 degree centigrade and CAMP test identified by by different biochemical tests.

Results: A total of 9(2.57%) isolates of *Listeria monocytogenes* were obtained from 350 samples of goat raw milk. All the isolates of *Listeria monocytogenes* were beta haemolytic and positive for CAMP reaction. All the isolates were negative for phenyl alanine deaminase, ornithine decarboxylase, lysine decarboxylase, malonate utilization and beta galactosidase tests. These were also negative for acid production from arabinose, D-xylose, mannitol, soluble starch and sucrose but acid was produced in rhamnose, salicin, and trehalose. Hydrogen sulfide production was recorded in tripticase soy broth with lead acetate paper strips but negative with triple sugar iron agar. Out of 9 isolates of *L. monocytogenes* only one produced acid from lactose. In serotyping all the isolates were serotype 4b and pathogenic in mice.

Conclusion: We can conclude that raw goat milk is contaminated with *L. monocytogenes* and can be harmful for human and animals.

Board No: 66 *(New and Re-) Emerging Zoonotic Diseases*

ALTERED GROWTH KINETIC AND CELL TROPISM FOR THE 2009 PANDEMIC H1N1 BY SINGLE REASSORTMENT OF THE NS SEGMENT

A. Mostafa[1,2], H. Petersen[3], B. Selvakumar[4], S. Herold[4], S. Rautenschlein[3], S. Pleschka[1]

[1]Institute of Medical Virology, Justus Liebig University Giessen, Giessen, Germany, [2]Center of Scientific Excellence for Influenza Viruses, National Research Center (NRC), Giza, Egypt, [3]Clinic for Poultry, University of Veterinary Medicine Hannover, Hannover, Germany, [4]Department of Internal Medicine Ii, Section of Infectious Diseases, University Hospital Giessen/Marburg, Giessen, Germany

Since the appearance and wide spread of the pandemic H1N1 influenza A virus (H1N1pdm09) in 2009, there is a main concern about the possibility of reassortment between such viruses and circulating highly pathogenic avian influenza viruses (HPAIVs). This concern is in part derived from the fact that H1N1pdm09 can infect turkey and possibly other bird species and could therefore recombine with avian influenza viruses and might have unexpected and unknown implications for animal and human health. By reverse genetics we explored the effect of NS segment reassortment between the H1N1pdm09 strain A/Giessen/06/09 (Gi-H1N1) and other human H1N1 influenza A virus, HPAIV of H5- and H7-subtypes and low pathogenic avian influenza virus (LPAIV) of the H7- and H9-subtype on the viral characteristics. We noticed a significant promotion in growth kinetic and change in host cell tropism of reassortant virus with NS segments of A/FPV/Rostock/34 (H7N1) and MDCK-adapted reassortant strain harboring NS segment of A/Puerto Rico/8/34 (H1N1) in secondary human, avian and porcine cell culture and in primary avian tracheal organ cultures. The Gi-H1N1 reassortants with NS segments of A/Wilson Smith N/33 (H1N1), A/Goose/Guangdong/1/96 (H5N1), A/Thailand/KAN-1/2004 (H5N1), A/Mallard/NL/12/2000 (H7N3), A/FPV/Bratislava/79 (H7N7), A/Anhui/1/2013 (H7N9) and A/Chicken/Saudi Arabia/CP7/98 (H9N2) showed replication efficiencies comparable to the parental H1N1-Gi. While, the NS segment of A/Victoria/3/75 (H3N2) reduced the replication efficiency of the Gi-H1N1 in different secondary cell lines. These results confirm that possible reassortment of H1N1pdm09 with different influenza A viruses should be continuously monitored and furthermore investigated *in vitro* and *in vivo* to predict the implications of such reassortant viruses for animal and human health.

Board No: 67 *(New and Re-) Emerging Zoonotic Diseases*

MATRIX-ASSISTED LASER DESORPTION IONIZATION—TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)-BASED RAPID SPECIES IDENTIFICATION WITHIN THE *STAPHYLOCOCCUS INTERMEDIUS-GROUP* (SIG)

J. Murugaiyan[1], B. Walther[2], I. Stamm[3], Y. Abou-Elnaga[4], S. Brueggemann-Schwarze[4], S. Vincze[4], L.H. Wieler[4], A. Lübke-Becker[4], T. Semmler[4], U. Roesler[1]

[1]Freie Universität Berlin, Centre for Infection Medicine, Institute of Animal Hygiene and Environmental Health, Berlin, Germany, [2]Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany, [3]Vet Med Labor Gmb H, Division of Idexx Laboratories, Ludwigsburg, Germany, [4]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany

Among coagulase-positive staphylococci (CPS) of companion animal origin, staphylococci composing the *Staphylococcus intermedius*-group (SIG) are common opportunistic pathogens, capable of causing a wide range of different purulent and toxin-mediated diseases in dogs, cats and horses. While there is a rising rate of methicillin-resistant *S. pseudintermedius* (MRSP) among microbiological specimens noticeable, phenotypic species identification techniques for the distinct members of the SIG might be more or less imprecise and time-consuming. In recent years, first severe infections with multi-drug resistant MRSP were also reported for humans. A fast and reliable identification of SIG, for instance by use of matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS), is necessary to disclose sound identification rates in both veterinary and human medicine.

As a first step, a reliable reference database for spectra associated with the different members of the SIG was created using isolates unambiguously identified by gene-based methods. A total of 27 MALDI-TOF MS spectra were acquired for each isolate of the following species: *S. pseudintermedius* (n = 43, including 20 MRSP), *S. intermedius* (n = 5) and *S. delphini* (n = 12) and a reference library was set up with Bruker Microflex LT together with BIOTYPER 3.0 software (Bruker Daltonics, Bremen, Germany). In a second approach, a broad convenience sample consisting of 200 CPS strains was evaluated using a) the original database content and b) the database after extension with distinct hierarchical clustered reference spectra for 60 SIG.

A significant improvement (average rise of log score value: 0.24) of the SIG identification score values was obtained for 200 SIG. Moreover, for 17 isolates the initial identification as "*S. intermedius*" changed to "*S. pseudintermedius*" as best match with improved score values by applying the in-house reference spectra extended database version.
Data presented here highlights the opportunities of sequence-based refinement of the Bruker database content with respect to improvement of MALDI-TOF MS-based bacterial species identification.

Board No: 68 *(New and Re-) Emerging Zoonotic Diseases*

GENERATION OF A MIDDLE EAST RESPIRATORY VIRUS (MERS) REVERSE GENETICS SYSTEM

A. Nikiforuk[1], A. Leung[2], D. Court[1], D. Kobasa[2], S. Theriault[1,3]

[1]Dept. Microbiology, University of Manitoba, Winnipeg, Canada, [2]Special Pathogens: Respiratory Viruses, Pathogenesis and Therapeutics, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada, [3]Applied Biosafety Research Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada

Background: *Coronaviridae* have significantly impacted public health on two occasions, in 2003 a coronavirus caused severe acute respiratory syndrome (SARS) and in 2012 a novel coronavirus emerged named Middle East respiratory syndrome (MERS). The size of the average coronavirus genome and their limited introduction into humans has made the study of their molecular characteristics and evolution difficult. Reverse genetic approaches have been used effectively in the past to molecularly and functionally characterize viruses (ex: *paramyxoviridae* and *filoviridae*). The recent emergence of MERS has made the development of MERS reverse genetics systems (RGS) a priority as they may be utilized for viral characterization, the development of animal models and designing prophylaxis.

Aim: To generate a synthetic MERS RGS utilizing the homologous recombination pathway of yeast which naturally corrects double stranded deoxyribonucleic acid break repair. Construction mediated by homologous recombination surpasses traditional cloning techniques in efficiency and speed. Therefore, successful assembly of a MERS RGS by homologous recombination would serve as a model approach for making future RGS of emerging viruses with large and complex genomes.

Methods: Approximately one-kilo base fragments of the MERS genome (JX869059.2) were synthesized by Eurofins genomics and used to construct a full-length MERS reverse genetics system. Methods included traditional and contemporary cloning techniques: polymerase chain reaction, splice by overlap extension polymerase chain reaction, T4 ligation, infusion ligation and the use of genetic assembly in the eukaryotic species *Saccharomyces cerevisiae*.

Results: Sanger sequencing and polymerase chain reaction of *S.cervisae* colonies has shown positive for the presence of the MERS-RGS. The MERS-RGS has been successfully shuttled from a eukaryotic host organism to a prokaryote, for ease of amplification and isolation. The MERS RGS has been used to rescue the MERS virus in a level 3 laboratory and the viral kinetics have been evaluated in comparison with the wilt-type MERS virus.

Conclusion: The development of a MERS-RGS allows virologists to conduct further work on the virus in Canada, possibly leading to the development of a vaccine or post exposure treatment.

Board No: 69 *(New and Re-) Emerging Zoonotic Diseases*

FORMATE HYDROGENLYASE ACTIVATOR (FHLA) ENHANCES RPON REGULATED GENE EXPRESSION IN *LEPTOSPIRA INTERROGANS*

C.J. Pappas[1,2], W. Hu[3,4], J. Zhang[3], Y. Yang[3], J. Yan[4], M. Picardeau[2], F. Yang[3]

[1]Biology, Manhattanville College, Purchase, United States, [2]Unite De Biologie Des Spirochetes, Institut Pasteur, Paris, France, [3]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, United States, [4]Department of Medical Microbiology and Parasitology, Zhejiang University School of Medicine, Hangzhou, China

Introduction: Leptospirosis is an emerging zoonotic disease transmitted via contaminated water sources. It is predicted that more than one million people are infected with leptospira, and that the mortality rate is greater than ten percent. *fhlA* is a putative enhancer binding protein in leptospires that may aid in gene specific activation of RpoN-RNA polymerase. RpoN has been shown to be involved with nitrogen assimilation and mammalian phase adaptation in bacteria.
Methods: Two distinct *fhlA* mutants (fhlA-1 & fhlA-2) were generated via random transposon mutagenesis in the pathogen *Leptospira interrogans*. *fhlA* complements (fhlA-/+1 & fhlA-/+2) were generated in fhlA- 1 with native fhlA under the control of *flgB*, a constitutively expressed promoter, and cis inserted into the genome.
Results: Morphological analysis demonstrated that loss of fhlA expression resulted in decreased cell length (WT=10.99 µm; fhlA-1= 6.07 µm; fhlA-2= 7.56 µm; fhlA-/+1= 10.11 µm; fhlA-/+2= 10.47 µm: WT vs. FhlA-1 or FhlA-2, $P<0.001$; FhlA-1 vs. fhlA-/+1 or fhlA-/+2, $P< 0.001$). Preliminary transcriptional analysis revealed specific genes under RpoN regulation have decreased expression in the absence of fhlA-1, and restored expression in fhlA-/+2 (*fhlA*, *lmanV1_2600001*, *lmanV1_4140003*). Intra-peritoneal inoculation of gerbils with fhlA-1 or fhlA-2 demonstrated virulence comparable to wild type.
Conclusion: FhlA plays a role in gene expression through RpoN in *L. interrogans*, and in part affects the general morphology of the spirochete. While *fhlA* does not appear to play a role in dissemination of the spirochete in gerbils, its role in infection remains unclear. Future intraocular inoculation of gerbils with *fhlA*- may reveal an *in vivo* phenotype.
This work was supported in part by National Science Foundation grant IIA-1159099
**C. Pappas and W. Hu contributed equally to this project.*

Board No: 70 *(New and Re-) Emerging Zoonotic Diseases*

SWINE INFLUENZA H3N2 VIRUS INFECTION IN AN IMMUNOCOMPROMISED ADULT PATIENT, ITALY 2014

E. Percivalle[1], A. Piralla[1], A. Moreno[2], M.E. Orlandi[3], C. Chiapponi[4], E. Foni[5], M. Luini[6], F. Baldanti[1]

[1]Molecular Virology Unit, Irccs Fondazione Policlinico San Matteo, Pavia, Italy, [2]Department of Virology, Istituto Zooprofilattico Sperimentale Della Lombardia Ed Emilia Romagna, Brescia, Italy, [3]Hematology Hunit, Irrcs Fondazione Policlinico San Matteo, Pavia, Italy, [4]Oie Reference Laboratory for Swine Influenza, Istituto Zooprofilattico Sperimentale Della Lombardia Ed Emiala Romagna, Parma, Italy, [5]Oie Reference Laboratory for Swine Influenza, Istituto Zooprofilattico Sperimentale Della Lombardia Ed Emilia Romagna, Parma, Italy, [6]Virology and Microbiology, Istituto Zooprofilattico Della Lombardia Ed Emilia Romagna, Lodi, Italy

Zoonotic infections in humans caused by swine influenza viruses (SIVs) have been seldom reported in Europe. At present, three influenza A subtypes (H1N1, H1N2 and H3N2) circulated in swine flocks in Italy. A human case of infection with an European swine H3N2 influenza virus is described.
On January 14, 2014, a 67-years male with multiple myeloma underwent routine follow-up visit at the Hematology Unit of the Fondazione IRCCS Policlinico San Matteo. The patient presented with upper respiratory tract infection and a nasal swab sample was positive for influenza A by real-time RT-PCR (6x106 RNA copies/ml). However, attempts to subtype the strain using real-time RT-PCR assays specific for human influenza H1, H3 and avian influenza H7N9 viruses were unsuccessful. The clinical sample was inoculated onto a mixed cells (Mv1Lu and A549 cells) monolayer and after 48h of incubation it was scored as positive using a monoclonal antibody (MAb) specific for influenza A/H3 antigen (Millipore, Billerica, USA).
The virus strain (A/Pavia/07/2014) was then propagated in MDCK cell cultures and embryonated chicken eggs. Full-genome sequencing was obtained using a MiSeq platform (Illumina, CA, USA). Phylogenetic tree of the HA and NA genes showed that A/Pavia/07/2014 strain was closely related to the European H3N2 SIV. In addition, phylogenetic trees of PB1, PB2, PA, NP, M and NS genes showed that A/Pavia/07/2014 strain clustered within the European SIV lineage including H1N1, H1N2 and H3N2 SIV strains. The HA gene of A/Pavia/07/2014 strain is 567 aa in length and the aa composition of antigenic sites was identical to those of SIV H3N2 strains circulated in swine flocks in Italy during 2013. Hemagglutinin inhibition (HI) antibody assay was positive in 9/11 sera collected from pigs in a farm close to patient's house.

In detail, HI testing was positive (HI titer, 40) in 2/11 (18.2%) pigs sera for the H1N1, H1N2 and H3N2 strains, in 5/11 (45.5%) pigs sera with HI titer 40 for the H1N2 strain and 2/11 (18.2%) pigs sera with HI titer 80 for H1N2 strain. Only 2/11 (18.2%) pigs sera were negative in the HI testing. In addition, real-time RT-PCR was performed in five nasal swabs collected from the same pigs and all were negative. At control visit, on January 29, 2014, the patient's nasal swab was negative for respiratory viruses. Furthermore, none of the patient's family and farm workers has developed respiratory.
In conclusion, the virological and serological data suggested that local pigs were the source of human infection. However, the spread of the SIV strain remains limited to the immunocompromised patient. Finally, the surveillance of circulating SIVs as well as monitoring occupationally exposed worker are two important tools to prevent spread of potential pandemic viruses.

Board No: 71 *(New and Re-) Emerging Zoonotic Diseases*

ACQUISITION OF ESBLS IN *SALMONELLA* SPP. AND *SHIGELLA* SPP. UNDER SYSTEMIC CEPHALOSPORIN THERAPY

B. Spellerberg[1], Y. Pfeifer[2], M. Pietsch[2], C. Denzer[3], C. Posovszky[3], A. Essig[1], W. Rabsch[4]

[1]Institute of Medical Microbiology and Hygiene, University Hospital Ulm, Ulm, Germany, [2]Nosocomial Pathogens and Antibiotic Resistances, Robert Koch Institute, Wernigerode, Germany, [3]Department of Paediatrics and Adolescent Medicine, University Hospital Ulm, Ulm, Germany, [4]National Reference Centre for Salmonella and Other Bacterial Enterics, Robert Koch Institute, Wernigerode, Germany

Salmonella and *Shigella* are a widespread cause of foodborne gastrointestinal infections. Here we report on emergence of extended-spectrum beta-lactamases (ESBLs) in these species isolated from three patients under cephalosporin therapy.

Patient 1 was a 15 year old boy who with a presumed exacerbation of ulcerative colitis (UC). Patient 2 was a 21 months old infant with diarrhoea due to a foodborne disease, and patient 3 was a 4 year old neuroblastoma patient with gastroenteritis. In all patients several stool samples were screened for enteric pathogens. Species identification, antimicrobial susceptibility testing and *Salmonella* serotyping were performed. Beta-lactamase genes were identified by PCR and sequencing. ESBL gene transfer was tested by broth mate conjugation experiments.

In patient 1 we found 5 isolates: *Salmonella* serovar Shubra (n=3), *E. coli* (n=1) and *Aeromonas hydrophila* (n=1). Culture of samples from patient 2 resulted in *Shigella boydii* (n=3) and *E. coli*(n=1). In patient 3 we found *Salmonella* serovar Manhattan (n=2), *E. coli* (n=2), *Citrobacter freundii* (n=2) and *Klebsiella pneumoniae* (n=1). The initial *Salmonella* and *Shigella* isolates of all patients were susceptible to 3rd generation cephalosporins. Thus, the patients received systemic cephalosporin treatment. However, all isolates from samples collected 6 days (patient 1), 9 days (patient 2) and 6 weeks (patient 3) later showed the ESBL-phenotype. We identified CTX-M-14 in *S.* Shubra, *E. coli* and *A. hydrophila* from patient 1. For patient 2 we found CTX-M-15-producing*S. boydii* and *E. coli.* Patient 3 was positive for SHV-12-producing *S.* Manhattan, *C. freundii*, *E. coli* and *K. pneumoniae*. ESBL genes *bla*CTX-M-14 and *bla*SHV-12 were located on conjugative plasmids of 65 kb and 50 kb size, respectively. Isolates of patient 2 harboured a 100 kb plasmid carrying *bla*CTX-M-15.

In all three cases the ESBL gene carrying plasmids in *Salmonella* and *Shigella* isolates were identical to plasmids in other ESBL-producing enterobacterial species that were present in the gut. Antibiotic treatment may enhance the selection of pathogens harbouring the resistance gene carrying plasmid. These cases demonstrate exemplarily the importance of *in vivo* plasmid transfer for the spread of antibiotic resistance genes among gram-negative bacteria of the human microbiome.

Board No: 72 *(New and Re-) Emerging Zoonotic Diseases*

NOVEL POLYOMAVIRUSES IN RAT (RATTUS NORVEGICUS) AND PIG (*SUS SCROFA*)

H. Preugschas, S. Korup, B. Ehlers

Fg12, Robert Koch Institute, Berlin, Germany

Polyomaviruses (PyVs) are a family of small, non-enveloped viruses with a circular double-stranded DNA that encode transforming proteins (T antigens). Thirteen human PyVs have been identified and some have been associated with human disease, including skin cancer caused by the recently discovered Merkel cell polyomavirus. Furthermore, it has been shown that some human PyVs induce tumors in foreign hosts (small laboratory rodents). On the other hand, the knowledge on non-human PyVs is limited to few animal species and it has not been investigated, if certain animal PyVs infect humans and eventually exhibit oncogenic properties. To identify hitherto unknown animal polyomaviruses, we used a combined approach of initial PyV identification with generic PCR, followed by long-range PCR and complete genome sequencing. Mammals with environmental proximity to humans were tested, and novel PyVs were identified in rats (Rattus norvegicus) and a pig (Sus scrofa). They were named Rattus norvegicus PyV 1 (RnorPyV1) and Sus scrofa PyV 1 (SscrPyV1). Both viruses were completely amplified and sequenced and encoded proteins determined. Possible splice variants of the T antigens were predicted and are currently confirmed in cell culture. To further investigate the prevalence of RnorPyV1 and SscrPyV1 within their natural host, organ samples from different origins were tested with specific PCR targeting the major capsid protein VP1. The VP1 of both PyVs was expressed in *E. coli* for use as antigen in enzyme-linked immunoassay (ELISA) and determination of seroprevalence.

Board No: 73 *(New and Re-) Emerging Zoonotic Diseases*

RELATIONSHIP BETWEEN ANTHRAX PROTECTIVE ANTIGEN (PA) IG G AND CLINICAL MANIFESTATIONS IN A PROSPECTIVE COHORT STUDY OF ANTHRAX INFECTION IN INDONESIA

D. Redhono[1], G. Hermawan[1], P. Dirgahayu[2]

[1]Internal Medicine, Sebelas Maret University/moewardi Hospital, Surakarta, Indonesia, [2]Biomedical Laboratory, Sebelas Maret University, Surakarta, Indonesia

Background: Anthrax is a zoonotic infectious disease problems that often occur in endemic regions. Risk factors for transmission of the most frequent is the contact of the animal or animal products infected with anthrax. Anthrax Protective Antigen (PA) Ig G is one screening test if there is an outbreak of anthrax in an area.
Method: We conducted a prospective cohort study, based Anthrax Protective Antigen Ig G after outbreaks of the clinical manifestations of anthrax in people exposed to anthrax in Indonesia from January through December 2012.
Result: Anthrax outbreak that occurred involving 100 people were exposed to anthrax, with the highest age distribution was 31 to 40 years as much as 42 %, and most were female gender. Test results showed serum Ig G antibody negative 50 %, borderline 15 % and 35 % positive. Risk factors of contact with animals suffering from anthrax is 34 % cooking and eating, 30 % eat meat and butchering and eating 24 %. Sixty percent of Ig G positive anthrax in people with risk of washing meat anthrax infected animals, the risk of slaughtering and eating meat 36 % showed positive results. The relationship between contact and Ig G results showed no significant relationship p = 0.078. Clinical manifestations that occur are antrak skin (4.2 %) one year post- PA titers obtained outbreaks 5 % positive and 85 % negative. Three percent of the results were still positive clinical manifestations in the skin occurs. The relationship between Ig G titers with clinical manifestations of anthrax at one year post- outbreak is highly significant p = 0.028.
Conclusion: Anthrax Protective Antigen Ig G was detected after one year post outbreaks of anthrax and meaningful relationship to clinical manifestations.

Board No: 74 *(New and Re-) Emerging Zoonotic Diseases*

PROTECTIVE FACTORS FOR SPORADIC HUMAN SALMONELLOSIS? RESULTS FROM A MATCHED CASE-CONTROL STUDY IN LOWER SAXONY, GERMANY, 2011-2013

S. Rettenbacher-Riefler[1], D. Ziehm[1], L. Kreienbrock[2], A. Campe[2], J. Dreesman[1]

[1]Mikrobiologie, Infektionsschutz, Krankenhaushygiene, Niedersächsisches Landesgesundheitsamt, Hannover, Germany, [2]Department of Biometry, Epidemiology and Information Processing, University for Veterinary Medicine, Hannover, Germany

Salmonella has long been recognised as an important food-borne zoonotic pathogen of economic significance in animals and in humans. For the majority of human salmonelloses the source of infection remains unclear. To investigate potential risk factors for sporadic salmonellosis, we invited notified cases and each four population controls matched on age, sex and geographical region to participate in a case-control study based on self-administered questionnaires.
Analysis of 285 matched pairs via conditional logistic regression revealed significant associations between sporadic salmonellosis and raw ground pork consumption (OR 6.0; 95% CI 2.2-20.1), intake of antacids (OR 5.8; 95% CI 1.4-24.5), eating meat at a restaurant (OR 5.7; 95% CI 2.2-14.6) and daily change or cleaning of dishcloth (OR 2.1; 95% CI 1.2-3.9). Animal contact 3 days prior to the disease as well as ice-cream consumption were negatively associated with the disease (OR 0.5; 95% CI 0.2-1 and OR=0.3; 95% CI 0.1-0.6, respectively). Infections with S. Typhimurium were significantly associated with raw ground pork consumption (OR= 15.4; 95% CI: 1.4-176.1) and S. Enteritidis infections were signifcantly associated with having travelled abroad (OR=5.4; 95% CI 1.0-28.3).
Our results corroborate previously identified risk factors, some of them being specific for certain salmonella subtypes. Furthermore, our data suggest that hygienic behaviour might increase and exposure to animals or consumption of ice-cream might decrease the risk of infection. We carefully assess the plausibility of these assumptions, as the causality between these exposures and salmonella infection is still ambiguous.
We conclude that it is most conceivable that case persons overestimated their hygiene behaviour retrospectively. Although a certain stimulating effect of regular animal contact on human immune defence might appear biologically plausible, pet owners have previously been described to visit their physician less often, which might have resulted in underreporting in the current study.

As there are at present no valid theories, we consider the negative association between salmonellosis and ice-cream consumption as a statistical artefact. Our findings illustrate that a sound interpretation of associations found in case-control studies as risk factors or protective effects also requires knowledge about microbiology and pathology of an infectious agent.

Board No: 75 *(New and Re-) Emerging Zoonotic Diseases*

OCCURRENCE OF CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE (CPE) ISOLATED FROM PIG-FATTENING FARMS THROUGHOUT GERMANY

N. Roschanski[1], C. von Salviati[1], J. Hering[2], A. Friese[1], B. Guerra[3], A. Käsbohrer[3], L. Kreienbrock[2], U. Rösler[1]

[1]Institute for Animal Hygiene and Environmental Health, Free University Berlin, Berlin, Germany, [2]Institute for Biometrics, Epidemiology and Information Processing, University of Veterinary Medicine Hannover, Hannover, Germany, [3]Department of Biological Safety, Federal Institute for Risk Assessment, Berlin, Germany

Carbapenems belong to the group of broad-spectrum beta-lactam antibiotics and are mostly considered as drugs of last choice for the treatment of serious infections in humans. Therefore, the dissemination of carbapenem resistance among Enterobacteriaceae possesses an increasing threat to public health. Although a wide variety of carbapenemase variants have been isolated from cases of human infections, until now just a few studies have reported their occurrence in livestock and livestock associated surroundings. However, since recently Enterobacteriaceae carrying *bla*VIM-1 genes have been isolated on a pig-farm in Germany (Fischer et al., 2012; 2013) the monitoring of CPE in livestock became a major topic within the European Union (EFSA, 2013).

As carbapenem resistance in Enterobacteriaceae is often associated with extended-spectrum-beta-lactamase or AmpC beta-lactamase production (Birgy et al., 2012) we recently started the carbapenemase-screening of bacterial isolates sampled during the first period (2011-2013) of the national RESET project (www.reset-verbund.de). Within this study the occurrence of ESBL-/ AmpC-producing Enterobacteriaceae on livestock farms throughout Germany was investigated. The screening described here is focused on 239 pooled feces and boot swab samples, chosen from a cross-sectional study including 58 pig-fattening farms throughout Germany. Also included were the farms with the recently by Fischer et al. described *E. coli* and *S. enterica* carrying *bla*VIM-1 genes.

DNA of the previously isolated bacterial samples was used for a real-time PCR based screening for the carbapenemase genes *bla*VIM, *bla*KPC, *bla*NDM, *bla*OXA-48 and *bla*GES. Out of 58 investigated farms one showed *bla*VIM-positive boot swap and feces samples, which indicates a low amount of findings.

However, within a simultaneously performed phenotypical screening approach, using MacConkey agar plates containing 0.125 mg/l meropenem (EFSA, 2013), additional Enterobacteriaceae were detected. These isolates are currently further investigated.
The so far obtained results are of great interest and the ongoing investigations will be of great importance to get an insight into the spread of carbapenemase genes among bacteria isolated from livestock.

Fisher et al., (2012), JAC: *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm.
Fisher et al., (2013), JAC: *Salmonella enterica subsp. enterica* procucing VIM-1 carbapenemase isolated from livestock farms Research Letters.
EFSA Scientific Opinion on Carbapenem resistance in food animal ecosystems (2013).
Birgy et al., (2012), JCM: Phenotypic screening of carbapenemase associated ß-Lactamases in Carbapenem-Resistent Enterobacteriaceae

Board No: 76 *(New and Re-) Emerging Zoonotic Diseases*

ESBL-PLASMIDS AFFECT CHROMOSOMALLY ENCODED FEATURES IN *ESCHERICHIA COLI* OF PANDEMIC SEQUENCE TYPES ST131 AND ST648

K. Schaufler[1], C. Ewers[2], T. Semmler[1], D.J. Pickard[3], G. Dougan[3], L.H. Wieler[1], S. Guenther[1]

[1]Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Berlin, Germany, [2]Justus Liebig Universität Giessen, Institute of Hygiene and Infectious Diseases of Animals, Giessen, Germany, [3]Wellcome Trust, Sanger Institute, Hinxton, United Kingdom

Abundant numbers of clinical as well as extra-clinical *Escherichia coli* isolates produce extended-spectrum beta-lactamase (ESBL)-enzymes. Several ESBL-gene families are encoded on plasmids. This study investigated the influence of ESBL-encoding plasmids of *E. coli*, which carry additional non-resistance genes, on chromosomally encoded features. Wild-type (WT) ESBL-producing *E. coli* strains were phenotypically and genotypically compared to generated ESBL-plasmid-free variants (PCVs) of pandemic sequence types ST131 and ST648.

PCVs were generated from WT strains using a "curing" heat method to force the loss of the large ESBL-plasmid. Plasmid characterization, macrorestriction analysis, and whole-genome sequencing was performed to confirm plasmid loss and to analyze the clonal identity of WT and PCV strains. Strains were compared via several phenotypic assays, including biofilm formation, adhesion and motility capacity. Various metabolic features were tested using Omnilog®. Transformants, which contain the reintroduced ESBL-plasmid were then constructed from PCVs via electroporation to verify the results. RNA-sequencing was performed to detect up- and down- regulated plasmidic as well as chromosomally encoded genes.

Plasmid characterization confirmed the extraction of only the large ESBL-plasmid. Pulsed-field gel electrophoresis revealed the genetic identities of WT and PCV strains. WT and PCV strains showed significant differences in several phenotypic tests including long-term colonies and swimming capacity. A reversion of the phenotypic characteristics in ESBL-transformants was detected in most strains, meaning the differences were invertible through the reintroduced ESBL-plasmid. Omnilog® results pointed towards a similar metabolic behavior of WT, transformant and PCV strains.

RNA-sequence analysis detected several up-regulated, biofilm-related genes and down-regulated, motility-related genes in WT and transformant strains compared to the corresponding PCV.
Differences in several phenotypic tests, the reversibility in transformants and up- respectively down-regulated genes indicate that ESBL-plasmids influence chromosomally encoded features. Concurrently, compared to the corresponding PCV strain, WT and transformant strains did not experience any metabolic disadvantages by carrying the ESBL-plasmid.
ESBL-plasmids, which additionally encode non-resistance genes, might play a so far underestimated role concerning the pandemic success of certain ESBL-producing *E. coli* isolates.

Board No: 77 *(New and Re-) Emerging Zoonotic Diseases*

FREQUENCY OF ANTIBODIES IGG AND IGM AGAINST *CHLAMYDIA PSITTACI* IN OCCUPACIONALLY EXPOSED PEOPLE IN ANTIOQUIA (COLOMBIA-SOUTH AMERICA)

J. Segura-Caro[1,2], X. Usuga-Perilla[3,4], C. Álvarez-Florez[3], A. Ossa-Giraldo[4,3,1,5]

[1]Facultad De Ciencias De La Salud, Institución Universitaria Colegio Mayor De Antioquia, Medellín, Colombia, [2]Biociencias Research Group, Institución Universitaria Colegio Mayor De Antioquia, Medellín, Colombia, [3]Infettare Research Group, Universidad Cooperativa De Colombia, Medellín, Colombia, [4]School of Medicine, Universidad Cooperativa De Colombia, Medellín, Colombia, [5]Biociencias Research Group, Institución Universitaria Colegio Mayor De Antioquia, Medellín, Colombia

Psittacosis is an infectious disease that affects several animals like birds, coats, pigs, rabbits and cats, etcetera, as well as human being when infected animals transmit the bacteria to them. This disease is distributed around the world; different countries make an active surveillance of psittacosis, but other as Colombia-South America does not have surveillance programs or enough knowledge about its epidemiological data, there is only one previous study. The aim of our study was to determine the frequency of IgG and IgM antibodies against *Chlamidophila psittaci* in people occupationally exposed to birds in Antioquia (Colombia). To determine the serum antibodies, we used microimmunofluorescence technique from Vircell® , and we discriminated these antibodies from others against *Chlamydia pneumoniae* and *Chlamydia trachomatis*. Until now, we have evaluated 32 people; 12 of them have antibodies against the bacteria (37%). These preliminary results are the first evidence of this bacteria in Antioquia and the second in Colombia. It is necessary to continue this research and to do others studies in order to know the epidemiology of this bacteria and the disease caused by it in Colombian humans and animals.

CDC. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. 2012 March 16; 61(10):169-180.

Monsalve S, Miranda J, Mattar S. Primera evidencia de circulación de Chlamydophila psittaci en Colombia: posible riesgo de salud pública. Rev. salud pública. 13 (2): 314-326, 2011

CFSPH. The Center for Food Security & Public Healt. Psittacosis/Avian Chlamydiosis. 2009; [February 28, 2012]. [6 pages]. Available in: http://www.cfsph.iastate.edu/Factsheets/pdfs/psittacosis.pdf

ILRI Mapping of poverty and likely zoonoses hotspots Zoonoses Project 4 Report to Department for International Development, UK.

NASPHV. National Association of State Public Health Veterinarians. Compendium of Measures To Control Chlamydophila psittaci Infection Among Humans (Psittacosis) and Pet Birds (Avian Chlamydiosis); 2010 [April 10, 2012].
[17 pages]. Available in: http://www.nasphv.org/Documents/Psittacosis.pdf

Board No: 78 *(New and Re-) Emerging Zoonotic Diseases*

INTEGRATED HUMAN AND ANIMAL RAPID RESPONSE FOR AVIAN INFLUENZA IN INDONESIA

V. Setiawaty[1], M. Misriyah[2], M. Azhar[3], N.L.P Indi Dhamayanti[4], H.A. Pawestri[1], E.W. Wulandari[5], M. Sudomo[5], P. Multihartina[6]

[1]Department for Virology, National Institute of Health Research and Development, Jakarta, Indonesia, [2]Directorate of Zoonotic Disease, Center for Disease Control and Evirontment Health, Jakarta, Indonesia, [3]Directorate of Animal Health, Ministry of Agriculture, Jakarta, Indonesia, [4]Department for Virology, Indonesian Research Center for Veterinary Science, Jakarta, Indonesia, [5]Disease Surveillance and Epidemiology, Who Office in Indonesia, Jakarta, Indonesia, [6]Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, Jakarta, Indonesia

Background: Since the first case of avian influenza (AI) H5N1 causing human infection in mid June 2005, the Indonesian government through Directorate General for Disease Control and Environment Health (DG DCEH), Ministry of Health Republic of Indonesia took action in rapid response integrated with Directorate of Animal Health, Ministry of Agriculture. Indonesia is a high risk country with endemic AI H5N1 in poultry in 32 out of 34 provinces. Furthermore backyard poultry practices and exposure to live bird markets are other important risk factors for AI transmission in Indonesia. Integrated rapid response is aimed at early detection of AI H5N1 cases and new strains such as H7N9.

Materials and Methods: Laboratory strengthening is one of the main components in rapid response of AI H5N1 cases. Other components include improving AI surveillance through event based surveillance and Early Warning Alert and Response System (EWARS) as well as SARI sentinel sites, risk communication and community empowerment and improving capacity of AI referral hospitals for clinical management and infection control. Joint risk assessment and response between human and animal health sector has been conducted through joint field investigation and risk analysis by rapid response team at sub national level, followed by technical discussion at all levels as input for evidence based decision making for better AI control efforts.

Results: AI cases have been steadily decreased year by year since the peak in 2006 when 55 cases occurred, although the case fatality rate remains high. In 2013 - May 2014, there were four human confirmed H5N1 cases reported by Ministry of Health. The epidemiology and virology investigation and risk analysis was done with integration between the human and animal health sectors.

The integrated data from human and animal was shared to both ministries. The field investigation found that the human H5N1cases living near and regularly visit the live bird market and two cases had pet birds and free range backyard poultry surround the houses. Since the appeal for vigilance for H5N1, mid June 2005 through May 2014, there have been 196 suspected H5N1 cases and 164 fatal. Almost of the H5N1 cases had a history of close contact with poultry.

Conclusions: Recognizing the importance of the integrated activities among human and animal health as part of rapid response for avian influenza, the Indonesian government through all levels of the MoH and MoA worked together effectively for outbreak preparedness, surveillance and response in one health spirit.

Board No: 79 *(New and Re-) Emerging Zoonotic Diseases*

HANTAVIRUS OUTBREAKS IN GERMANY: VIRUSES AND HOSTS COME AND GO?

H. Sheikh Ali[1], V. Weber de Melo[2], R. Ryll[3], S. Drewes[3], M. Schlegel[3], K. Wanka[3], S. Essbauer[4], J. Freise[5], M. Groschup[3], G. Heckel[2,6], R. Ulrich[3]

[1]Innt, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany, [2]Institute of Ecology and Evolution, University of Bern, Bern, Switzerland, [3]Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut (FLI), Greifswald, Germany, [4]Department of Virology &Amp; Rickettsiology, Bundeswehr Institute of Microbiology, Munich, Germany, [5]Fachbereich Schädlingsbekämpfung, Task Force Veterinärwesen, Niedersächsisches Landesamt Für Verbraucherschutz Und Lebensmittelsicherheit, Wardenburg, Germany, [6]Swiss Institute of Bioinformatics, Genopode, Lausanne, Switzerland

Puumala virus (PUUV) is the predominant hantavirus species in Europe, causing mild to moderate cases of haemorrhagic fever with renal syndrome. Its negative stranded RNA genome consists of three segments. In Germany the bank vole Myodes glareolus represents the reservoir of PUUV. Lower Saxony is an endemic region for PUUV infections in Germany, and the district of Osnabrück presents the highest incidence of human PUUV infections within this area. Five localities in this district were monitored from 2005 to 2012. Phylogenetic and population genetic analyses of partial sequences of all three genome segments of PUUV strains revealed a spatial clustering of these sequences. The genetic structure of bank vole populations showed a temporally persisting subdivision at a geographic scale of 2.5 to 14 kilometres. The subdivision of the bank vole populations did not correspond to the virus populations. At each site there was a high temporal turnover of virus strains in the vole population, but several virus strains persisted through multiple years. The first complete genome sequence of a PUUV strain from this region showed a similarity to other PUUV strains of 80.1% to 84.7% at the level of the nucleotide sequence, and between 89.5% and 98.1% for the deduced amino acid sequences. In conclusion, the investigations demonstrated a continuing prevalence in an endemic region and a high sequence variability of PUUV in the local vole populations. The non-homogenous sequence variation within the genome segments may allow the development of RT-PCR assays for distinguishing PUUV strains at different spatial and temporal scales.

Board No: 80 *(New and Re-) Emerging Zoonotic Diseases*

IDENTIFICATION OF POTENTIAL BIOMARKERS FOR THE VIRULENCE OF *COXIELLA BURNETII* STRAINS

K. Sobotta[1], C. Heydel[2], K. Kerner[2], K. Hillarius[1], C. Menge[1]

[1]Infektionsimmunologie, Friedrich-Loeffler-Institut (FLI), Jena, Germany, [2]Institut Für Hygiene Und Infektionskrankheiten Der Tiere, Universität Gießen, Gießen, Germany

Differences in courses of infection due to the Q fever bacterium *Coxiella burnetii* (*C. b.*) point to strain specific virulence attributes. As macrophages are the pathogens' natural target cells the mutual impact of monocyte derived macrophages (MDM) from cattle and humans and *C. b.* isolates differing in origin and genotype was analyzed to identify biomarkers for *C. b.* virulence.

MDM were inoculated with either of 13 *C. b.* strains and replication rates calculated by determining the tissue culture infective dose 1, 7 and 14 days post inoculation (d. p. i.) by endpoint titration. Macrophage responses were assessed by quantifying mRNA of 10 cytokines 3 h p. i. via RT-PCR and 5 surface markers 1 d p. i. via flow cytometry.

By 14 d. p. i. replication rates of *C. b.* strains ranged from stagnation to excessive proliferation with growth factors up to 1,000 and 100,000 in bovine and human MDM, respectively. Host cell responses were quantitatively different but not correlated with the growth kinetics of C. b. strains. Overall, responses were characterized by a stronger up-regulation of Th1-associated cytokines than of Th2-associated cyto- and chemokines by all but one strain in bovine and human MDM. Strain 19/34 induced a weaker Th1 reaction in human MDM and a distinct increase in MCP1 transcription favouring Th2 response. All strains except Henzerling and Z6906 failed to induce expression of co-stimulatory molecules CD40 or CD80 in bovine MDM. Differences in replication and MDM response were not correlated with genetic properties (plasmid, MLVA, adaA status) of *C. b.* strains. Further investigations will aim to link genetic elements to a strain specific impact on the immune balance and may serve as a novel approach for estimating virulence of *C. b.* strains.

Board No: 81 *(New and Re-) Emerging Zoonotic Diseases*

KEMEROVO VIRUS DETECTION IN DIFFERENT REGIONS OF WESTERN SIBERIA

S. Tkachev[1], A. Tikunov[1], N. Livanova[1,2], S. Livanov[2], V. Panov[3], V. Yakimenko[4], A. Tantsev[4], I. Babkin[1], N. Tikunova[1]

[1]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russian Federation, [2]Laboratory of Zoomonitoring, Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russian Federation, [3]Laboratory of Ecology of Vertebrates' Communities, Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russian Federation, [4]Laboratory of Arbovirus Infections, Omsk Research Institute of Natural Foci Infections, Omsk, Russian Federation

In 1962 the expedition of Institute of poliomyelitis and virus encephalitis AMS USSR in collaboration with Kemerovo regional sanitary-hygienic station and Institute of virology from Czechoslovak Academy of Sciences under the direct leadership of Prof. M.P. Chumakov isolated and described Kemerovo virus (KEMV) from Ixodes persulcatus ticks and clinical samples in Kemerovo region of Western Siberia, Russia. It was demonstrated that this member of *Reoviridae* family can cause febrile forms of disease and affect the human central nervous system.

The aim of our work was KEMV RNA detection in ixodid ticks collected in different regions of Western Siberia (Novosibirsk Akademgorodok, Omsk region, Altai and Salair Ridge).

Using primers specific to Kemerovo viruses group genome segments 1 and 2 KEMV RNA was found in 1.5% of ticks from Novosibirsk region, in 2,3% of ticks from Omsk region, in 2,2% of ticks from Altai and in 13,3% of ticks from Salair Ridge. It should be noted that the ticks' infection rate with KEMV was higher than infection with tick-borne encephalitis virus in Salair Ridge territory. So, in the present study, KEMV RNA was detected for the first time in I. persulcatus ticks from all studied territories, as well as for the first time at all in I. pavlovskyi ticks from Novosibirsk region.

For some positive samples the sequences of KEMV genome segment 2 were determined. The phylogenetic analysis of sequences confirmed that all found virus isolates clusterized with Kemerovo group viruses and KEMV isolates found in Novosobirsk, Omsk and Kemerovo oblasts formed the separate clades on dendrogram.

This work was partially supported by the Siberian Branch of the Russian Academy of Sciences (Integration interdisciplinary project no. 135).

Board No: 82 *(New and Re-) Emerging Zoonotic Diseases*

THE GENETIC AND BIOLOGICAL PROPERTIES OF TICK-BORNE ENCEPHALITIS VIRUS UNIQUE GROUP FROM EASTERN SIBERIA

S. Tkachev[1], I. Kozlova[2,3], M. Verkhozina[4], T. Demina[5], Y. Dzhioev[2,6], L.S. Karan[7], E. Doroschenko[2], O. Lisak[2], O. Suntsova[2], A. Paramonov[2], V. Zlobin[3]

[1]Laboratory of Molecular Microbiology, Institute of Chemical Biology and Fundamental Medicine Sb Ras, Novosibirsk, Russian Federation, [2]Laboratory of Molecular Epidemiology and Genetic Diagnostics, Fssfe Scientific Centre of Family Health and Human Reproduction Problems Sb Rams, Irkutsk, Russian Federation, [3]Department of Microbiology, Virology and Immunology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [4]Department of Virology Research and Pcr Laboratory, Centre for Epidemiology and Hygiene in Irkutsk Region, Irkutsk, Russian Federation, [5]Faculty of Veterinary Medicine and Biotechnology, Irkutsk State Academy of Agriculture, Irkutsk, Russian Federation, [6]Laboratory of Molecular Virology and Biotechnology, Institute of Biomedical Technology, Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russian Federation, [7]Laboratory of Epidemiology of Natural Foci Infections, Central Research Institute of Epidemiology of Rospotrebnadzor Rf, Moscow, Russian Federation

According to the modern classification, tick-borne encephalitis virus (TBEV) is divided into three genotypes: Far-Eastern, Siberian and Western (European). In Eastern Siberia the circulation of three genotypes with the domination of Siberian genotype was described earlier.

In our study, with use of molecular hybridization of nucleic acids method with genotype-specific probes and sequencing of complete virus genome or its fragments we identified the group of 18 strains formed an independent branch on dendrogram and did not cluster with any strains of three main genotypes. These strains (called "group 886") were isolated from ticks and small mammals collected in Irkutsk region, Buryat Republic and Transbaikalia in 1984-1990. Also two "group 886" strains were described recently on the territory of Transbaikalia from taiga tick (in 1999) and one strain from *Myodes rutilus* (in 2010). Moreover, the case of meningoencephalitis with lethal outcome was described in Mongolia caused by TBEV isolate with genome fragment sequence similar to "group 886" strains.

The analysis of complete amino acid sequences of some strains polyprotein confirmed that it's the "mixture" of sequences common for three genotypes. Twenty-nine unique substitutions were detected which could probably be the "genotype-specific" for "group 886" members.

The studies of biological properties demonstrated that "group 886" strains have the wide spectrum of antigenic properties, hemagglutination and neutralizing activities, high virulence and thermotolerance.
So, the obtained data confirm that this TBEV variant could be the new genotype.
The study was partially supported by interdisciplinary integration project №135 of SB RAS.

Board No: 83 *(New and Re-) Emerging Zoonotic Diseases*

IDENTIFICATION OF THE SOURCE OF AN (H10N8) VIRUS CAUSING HUMAN INFECTION

Y. Xu[1], H. Cao[2], H. Liu[2], H. Sun[1], B. Martin[1], Y. Zhao[1], Q. Wang[2], G. Deng[2], J. Xue[1], Y. Zong[2], J. Zhu[2], F. Wen[1], L. Long[1], S. Wong[3], N. Zhao[1], X. Fu[4], M. Liao[5], G. Hu[2], R. Webby[3], G. Gao[6], X. Wan[1]

[1]Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, United States, [2]College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China, [3]Department of Infectious Diseases, St. Jude Children´s Research Hospital, Memphis, United States, [4]Jiangxi Provincial Center for Animal Disease Control and Prevention, Nanchang, China, [5]College of Veterinary Medicine, South China Agricultural University, Guangzhou, China, [6]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Academy of Sciences, Beijing, China

A novel influenza A (H10N8) virus has been detected in three humans in China since December 2013. Although this virus was hypothesized to be a novel reassortant between influenza viruses from wild birds and domestic poultry, its evolutionary path leading to human infection is unknown. Sporadic surveillance between April 2013 and January 2014 at the live poultry market (LPM) suspected to be the source of infection for the first H10N8 case showed a gradual increase in influenza virus prevalence had occurred culminating with a predominance of H10N8 viruses. Influenza viruses detected in the LBM up to 8 months prior to human infection contributed genetic components to the zoonotic virus. These H10N8 viruses have continued to evolve within this LBM subsequent to the human infection and continuous assessments of these H10N8 viruses will be necessary. Reduction of influenza virus burden in LPMs is essential in preventing future emergence of novel influenza viruses with zoonotic and pandemic potential.

Board No: 84 *(New and Re-) Emerging Zoonotic Diseases*

GENETIC BASIS OF RESISTANCE TO SPECTINOMYCIN AMONG STAPHYLOCOCCI FROM VARIOUS ANIMAL ORIGINS COLLECTED IN THE RESISTANCE MONITORING STUDY BfT-GermVet

S. Wendlandt, K. Kadlec, S. Schwarz

Friedrich-Loeffler-Institut (FLI), Institute of Farm Animal Genetics, Neustadt Mariensee, Germany

During the BfT-GermVet study, a total of 248 staphylococci from diseased pigs, dogs and cats were investigated for their susceptibility to numerous antimicrobial agents. Eleven staphylococcal isolates showed high MICs (≥512 mg/L) of spectinomycin, including four *S. hyicus* isolates from pigs, four *S. aureus* isolates from pigs (n=3) and a cat (n=1), two *S. intermedius* group isolates from cats as well as a single *S. simulans* isolate from a pig. The aim of the present study was to identify the genetic basis of spectinomycin resistance among these isolates.

The isolates were tested for the presence of the staphylococcal spectinomycin resistance genes *spc*, *spw* and *spd* by PCR. The plasmid location of spectinomycin resistance genes was tested by transformation into *S. aureus* RN4220 and Southern blotting. Restriction and/or sequence analysis were conducted to determine the plasmid types of the respective transformants.

The four *S. aureus* isolates and the two feline *S. intermedius* group isolates harboured spc. Single *S. hyicus* isolates carried the *spw* or *spd* gene. The remaining two *S. hyicus* isolates and the single*S. simulans* isolate carried the *spc* as well as the *spd* gene. All four *spd* genes were located on spectinomycin resistance plasmids with different restriction patterns and sizes of approximately 3.7-4.5 kb.

This is the first identification of the *spw* gene in a *S. hyicus* isolate and also *spd*-carrying plasmids in *S. hyicus* and *S. simulans*. Furthermore, this study showed that more than one spectinomycin resistance gene can be present in the same staphylococcal isolate.

Board No: 85 *(New and Re-) Emerging Zoonotic Diseases*

IDENTIFICATION OF THE NOVEL PLEUROMUTILIN RESISTANCE GENE *Lsa* (E) IN BOVINE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND COAGULASE-NEGATIVE STAPHYLOCOCCI

S. Wendlandt, K. Kadlec, A.T. Feßler, S. Schwarz

Friedrich-Loeffler-Institut (FLI), Institute of Farm Animal Genetics, Neustadt Mariensee, Germany

In recent years, combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in staphylococci has been attributed to ABC transporters of the Vga type. More recently, a novel gene of the *lsa* type has been detected and shown to confer this resistance phenotype. This gene was designated *lsa*(E) and has up to now been found in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* (MRSA/MSSA) of clonal complex (CC) 398 or CC9 of human, pig and poultry origin. The aim of this study was to investigate the genetic basis of pleuromutilin resistance in MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) isolates from dairy cows suffering from mastitis.

Among 47 methicillin-resistant staphylococcal isolates from cases of bovine mastitis, 27 bovine isolates were identified as tiamulin resistant. These 27 isolates, 14 MRSA isolates and 13 MRCoNS, were tested for the presence of the pleuromutilin resistance genes *vga*(A), *vga*(C), *vga*(E) and *lsa*(E) by specific PCR assays.

None of the 27 bovine isolates carried the *vga*(C) or *vga*(E) gene. four MRCoNS isolates were *vga*(A)-positive. Twelve MRSA isolates and two MRCoNS isolates carried the *lsa*(E) gene. Another four MRCoNS isolates were positive for both genes *vga*(A) and *lsa*(E). None of the so far known genes was detected in the remaining two MRSA and three MRCoNS.

To conclude, this is the first identification of the novel *lsa*(E) gene in bovine MRSA isolates and in MRCoNS. Moreover, this novel gene was detected very frequently with 14 of 27 tiamulin resistant isolates being positive for *lsa*(E).

Board No: 86 *(New and Re-) Emerging Zoonotic Diseases*

STAPHYLOCOCCUS AUREUS CC398 OF ANIMAL AND HUMAN ORIGIN: EASY DISCRIMINATION BETWEEN THE HUMAN ANCESTORS, THE ANIMAL ASSOCIATED SUBPOPULATION, AND ISOLATES OF SECONDARY HUMAN ORIGIN

C. Cuny, M. AbdElBary, F. Layer, G. Werner, W. Witte

Wernigerode Branch, Robert Koch Institute, Wernigerode, Germany

Objective: As known from comparative genome analysis S.aureus C398 originated from humans, accompanied by acquisition of mecA a subpopulation became widely disseminated among livestock and other animals (mainly as colonizer and came back to humans where it is not only a colonizer but also able to cause various kinds of infections. Here we report on discrimination between the ancestral (human adapted) clade and the subpopulation (animal and secondary human origin) by ordinary PCR for the immune evasion gene cluster (IEC) and for the SNP in gene SAPIG 0689.
Material and Methods: MRSA CC398; 96 isolates from different kinds of infections in humans, 48 isolates from nasal colonization of pigs, 16 isolates from horses. MSSA CC398; 15 isolates from infections in humans. PCR for IEC was performed according to (1), for detection of the SNP in SAPIG 0 by which the ancestral clade and the subpopulation can be discriminated (2) we designed degenerated primers.
Results: 17 among the 96 MRSA (SCCmecV) isolates from infections in humans contained IEC, 1 of them was attributed to the ancestral cluster. 9 among the 15 MSSA belonged to the ancestral population and contained IEC, 6 isolates were negative for IEC and attributed to the animal subpopulation. All of the isolates from animals were attributed to this subpopulation and negatve for IEC.
Conclusion: discrimination between the ancestral clade of S.aureus CC398 is easily possible by ordinary PCR for the SNP in SAPIG0689 , If positive, PCR for IEC indicates secondary human origin.

Verkaik NJ, et al. Clin Microbiol Infect. 2011:343-8.
Stegger, M. e tal. PLoS One. 2013 Nov 14;8(11):e79645. doi:

Board No: 87 *(New and Re-) Emerging Zoonotic Diseases*

STREPTOCOCCUS SUIS AFFECTS THE REPLICATION OF SWINE INFLUENZA VIRUS IN PORCINE TRACHEAL CELLS

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

[1]Institute of Virology, University of Veterinary Medicine Hannover, Hannover, Germany, [2]Institute of Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany

Swine influenza viruses (SIV) are important pathogens affecting pigs of all ages. Surveillance data show that 31% of European pig farms are exposed to SIV. *Streptococcus suis* is one of the most important bacterial respiratory pathogens in the swine population. Secondary infection by *S. suis* may enhance the severity of disease in piglets infected by SIV resulting in huge economic losses. To date, the relationship between SIV and *S. suis* still remains unclear. In order to understand the interaction between SIV and *S. suis*, we established an *in vitro* co-infection model based on newborn pig trachea cells (NPTr). Two SIV variants A/sw/Bad Griesbach/IDT5604/2006 H1N1 and A/sw/Herford/IDT5932/2007 H3N2 were used to compare subtype differences. Our previous studies showed that this H3N2 strain had a higher replication rate and induced a strong ciliostatic effect in pig precision-cut lung slices, while the H1N1 strain only had a mild effect on the ciliary activity. Wild type *S. suis* (WT) and an noncapsulated mutant strain (Δcps) were selected as secondary bacterial pathogens in this study. NPTr cells were infected with different combinations, first inoculated with SIV, followed by bacterial inoculation. The course of infection was monitored by immunofluorescence microscopy and by determining the virus titers at different time points. After SIV infection, the adhesion rate of WT bacteria was enhanced. Different from Δcps, most of the WT bacteria adhered to SIV infected cells. Furthermore, the viral replication rates of both H1N1 and H3N2 SIV were reduced when cells were co-infected by *S. suis* WT strain. Interestingly, the virus titers in WT co-infected groups were ten-fold lower than in SIV mono-infection or Δcps co-infected groups at 24 h.p.i. These results indicate that *S. suis* and SIV affect each other in the infectious behavior in our NPTr cell model.

Board No: 88 *(New and Re-) Emerging Zoonotic Diseases*

THE ADAPTATION OF AVIAN INFLUENZA VIRUSES TO THE RESPIRATORY EPITHELIUM OF PIGS

W. Yang[1], F. Meng[1], D. Punyadarsaniya[2], M. Hoffmann[1], J. Stech[3], D. Hoeper[3], M. Beer[3], C. Schwegmann-Wessels[1], X. Ren[4], G. Herrler[1]

[1]University of Veterinary Medicine, Hannover, Institute of Virology, Hannover, Germany, [2]Mahanakorn University of Technology, Institute of Virology, Bangkok, Thailand, [3]Bundesforschungsinstitut Für Tiergesundheit, Friedrich Loeffler Institut, Greifswald Isle of Riems, Germany, [4]Northeast Agricultural University, College of Veterinary Medicine, Harbin, China

Pigs are an important host for influenza A viruses and may play a crucial role in the interspecies transmission. To analyze the infection by influenza viruses, we have established precision-cut lung slices from the porcine lung as a culture system for differentiated respiratory epithelial cells. As differentiated repiratory epithelial cells are the primary target cells for influenza virus infections, precision-cut lung slices provide an interesting system to analyze the adaptation of avian influenza viruses to the respiratory epithelium of pigs. Avian influenza viruses H9N2 subtype have been circulating worldwide in multiple avian species and have repeatedly infected mammalian to cause typical disease. The continued avian-to-mammalian interspecies transmission of H9N2 viruses raises concerns about the possibility of viral adaption with increased virulence for humans and poses a potential health risk to the public. Avian influenza viruses H9N2 subtype were subjected to several passages in precision-cut lung slices. Then the changes in the viral properties that are associated with the adaptation process were characterized by analyzing: (1) duration of the growth cycle; (2) amount of infectious virus released into the supernatant; (3) extent of the ciliostatic effect. Sequence analysis will reveal which amino acid changes occur during the different virus passagesAdaptation of the avian viruses to growth in porcine cells was evident in a shortening of the growth cycle. Sequence analysis revealed that few amino acid changes occurred during the different virus passages. The importance of the individual mutations is currently analyzed by generating recombinant viruses that contain the respective mutated proteins. Our study will help to understand the processes involved in the adaptation of H9N2 influenza viruses to new hosts.

Board No: 89 *(New and Re-) Emerging Zoonotic Diseases*

PRESENCE OF ANTIBODIES TO TICK BORNE ENCEPHALITIS VIRUS IN CHILDREN WITH ARTHRITIS IN TURKEY

H. Yilmaz[1], A. Karakullukcu[2], O. Kasapcopur[3], A.A. Ozkul[4], E. Altan[1], U. Cizmecigil[1], M.U. Esatgil[5], Z. Bilgin[5], A. Yilmaz[6], N. Turan[1], C. Klaus[7], J.A. Richt[8], B. Kocazeybek[2]

[1]Department of Virology, University of Istanbul, Veterinary Faculty, Istanbul, Turkey, [2]Department of Medical Microbiology, University of Istanbul, Cerrahpasa Medical School, Istanbul, Turkey, [3]Department of Child Health and Diseases, University of Istanbul, Cerrahpasa Medical School, Istanbul, Turkey, [4]Department of Child Health and Diseases, University of Izmir, Medical Park Hospital, Karsiyaka, Izmir, Turkey, [5]Department of Parasitology, University of Istanbul, Veterinary Faculty, Avcilar, Istanbul, Turkey, [6]Cevre Laboratories, Tatli Pinar Sokak, Mart Plaza, Kat 2, Kagıthane, Istanbul, Turkey, [7]Institut Für Bakterielle Infektionen Und Zoonosen, Friedrich Loeffler Institut, Federal Research Institute for Animal Health, Jena, Germany, [8]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, United States

Keywords: TBEV, ELISA, Arthritis, Children, Turkey

Tick borne encephalitis virus (TBEV) is mainly transmitted by ticks and infects vertebrated animals and people causing fever and occasional neurological disorders. The aim of this study was to investigate antibodies to TBEV in children with arthritis. For this, 90 sera were collected from children showing fever and/or symptoms of arthritis. Sera was analysed by ELISA kits (Euroimmun) for the presence of IgM and IgG antibodies to TBEV. IgM antibodies to TBEV were detected in 3 children between the age of 3-7 years old. No IgG antibodies to TBEV were detected. Children who had antibodies to TBEV had fever and arthritis but no neurological disorders. Results of this study indicate that children are exposed to TBEV infection in Turkey and it is important to follow up these children with arthritis for neurological disorders.

Board No: 90 *(New and Re-) Emerging Zoonotic Diseases*

PRELIMINARY SCREENING RESULTS OF MOSQUITOES FOR WEST NILE VIRUS (WNV) AND RIFT VALLEY FEVER VIRUS (RVFV) IN THE EUROPEAN BORDER REGION OF TURKEY (THRACE DISTRICT)

H. Yilmaz[1], Z. Bilgin[2], E. Altan[1], U. Cizmecigil[1], F.M. Simsek[3], M.U. Esatgil[2], A. Karakullukcu[4], N. Turan[1], A. Yilmaz[5], O. Kasapcopur[6], K. Barut[6], A.A. Ozkul[7], B. Faburay[8], B. Kocazeybek[4], E. Tuzer[2], J.A. Richt[8]

[1]Department of Virology, University of Istanbul, Veterinary Faculty, Istanbul, Turkey, [2]Department of Parasitology, University of Istanbul, Veterinary Faculty, Istanbul, Turkey, [3]Department of Ecology, University of Adnan Menderes, Faculty of Science and Literature, Division of Biology, Aydin, Turkey, [4]Department of Medical Microbiology, University of Istanbul, Cerrahpasa Medical School, Istanbul, Turkey, [5]Tatli Pinar Sokak, Mart Plaza, Kat 2, Kagıthane, Cevre Laboratories, Istanbul, Turkey, [6]Department of Child Health and Diseases, University of Istanbul, Istanbul, Turkey, [7]Department of Child Health and Diseases, University of Izmir, Medical Park Hospital, Izmir, Turkey, [8]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, United States

Keywords: WNV, RVFV, Mosquito, real-time RT-PCR, Turkey

West Nile virus (WNV) and Rift Valley fever virus (RVFV) are transmitted by mosquitos and infect vertebrate hosts including people causing fever and occasional deaths. The aim of this study was to screen mosquitos for the presence of WNV and RVFV-RNA in the European border region of Turkey (Thrace district). For this purpose, light traps were placed in the vicinity of Kırklareli, Edirne, Tekirdağ and their localities and left through midnight. The mosquitoes were identified by using microscopical observation. Among 6585 mosquitos collected, 3338 belonged to the *Culex* spp., 3128 to the *Anofel* spp., 75 to the *Aedes* spp., and 44 to the *Culicata* spp. RNA was extracted from pools of 5 to 30 mosquitoes and analyzed for the presence of WNV and RVFV-RNA by using a SYBR-Green real-time RT-PCR. So far, no WNV or RVFV-RNA was detected in the mosquito pools. Further analyses of mosquitoes from the border region are in progress.

LIST OF PARTICIPANTS

List of Participants

Name	Institute and e-mail
Abass, Naglaa GERMANY	Friedrich-Loeffler-Institut Naglaa77_Hakeem@yahoo.com
Abdelgawad, Azza GERMANY	Leibniz Institute for Zoo and Wildlife Research azza@izw-berlin.de
Abdiyeva, Karlygash GERMANY	Kazakh National Medical University karla.abdi@yandex.ru
Al Dahouk, Sascha GERMANY	Federal Institute for Risk Assessment sascha.al-dahouk@bfr.bund.de
Alsakee, Hadi IRAQ	Hawler Medical University hadialsakee@gmail.com
Andersson, Maria SWEDEN	Public Health Agency of Sweden maria.andersson@folkhalsomyndigheten.se
Andersson, Martin SWEDEN	Lund University Martin.Andersson@biol.lu.se
Angermeier, Hilde GERMANY	University of Wuerzburg Angermeier@stud.uni-heidelberg.de
Arnold, Ulrike GERMANY	Robert Koch Institute arnoldu@rki.de
Aurich, Gesche GERMANY	Institut für Med. Mikrobiologie, gesche.aurich@tum.de
Aydin, Mehmet Fatih TURKEY	University of Karamanoglu veterinermfa@gmail.com
Bakre, Hawri, IRAQ	Hawler Medical University dr_hawreb@yahoo.com

Name	Institute and e-mail
Balasubramaniam, Ramasamy SRI LANKA	Ministry of Health yobala27@gmail.com
Balkema-Buschmann, Anne GERMANY	Friedrich-Loeffler-Institut anne.buschmann@fli.bund.de
Ballhausen, Britta GERMANY	University Hospital Münster britta.ballhausen@ukmuenster.de
Banerjee, Swapan CANADA	Health Canada swapan.banerjee@hc-sc.gc.ca
Becher, Dietmar GERMANY	MICROMUN GmbH becher@micromun.de
Bechlars, Silke GERMANY	Federal Institute for Risk Assessment silke.bechlars@bfr.bund.de
Beer, Martin GERMANY	Friedrich-Loeffler-Institut silke.bechlars@bfr.bund.de
Beier, Ilka GERMANY	ilkabeier@hotmail.com
Beitrishvili, George Akaki GEORGIA	Infectious Disease Aids and Clinical Immunology Research Center georg.beit@gmail.com
Ben Said, Mourad TUNISIA	National School Veterinary Medicine of Sidi Thabet bensaidmourad83@yahoo.fr
Benacer, Douadi MALAYSIA	University of Malaya benacer.dvt@gmail.com
Bereswill, Stefan GERMANY	Charité - University Medicine stefan.bereswill@charite.de

List of Participants

Name	Institute and e-mail
Bethe, Astrid GERMANY	Free University Berlin astrid.bethe@fu-berlin.de
Bier, Nadja GERMANY	Federal Institute for Risk Assessment nadja.bier@bfr.bund.de
Blaňarová, Lucia SLOVAKIA	Institute of Parasitology of the Slovak Academy of Sciences blanarova@saske.sk
Boden, Katharina GERMANY	Institute of Laboratory Medicine, University Hospital Jena katharina.boden@med.uni-jena.de
Bonkowski, Katharina GERMANY	Friedrich-Loeffler-Institut katharina.bonkowski@fli.bund.de
Boone, Ides GERMANY	Federal Research Institute on Risk Assessment idesbald.boone@bfr.bund.de
Boots, Michael UK	mikeboots@gmail.com
Borovikov, Sergey KAZAKHSTAN	S. Seifullin Kazakh Agro- Technical University nicsb_katu@mail.ru
Böttcher, Sindy GERMANY	Robert Koch Institute BoettcherS@rki.de
Bourquain, Daniel GERMANY	Robert Koch Institute bourquaind@rki.de
Brandenburg, Anja GERMANY	Tierärztliche Praxis anja.g.brandenburg@t-online.de
Bröckl, Stephanie GERMANY	Bavarian Health and Food Safety Authority stephanie.broeckl@lgl.bayern.de

List of Participants

Name	Institute and e-mail
Bruchmann, Anja GERMANY	Mircomol GmbH a.bruchmann@micromol.com
Brückner, Sina GERMANY	Institute for Tropical Medicine University of Tübingen sinab@gmx.net
Bruhn, Silke SWITZERLAND	Federal Food Safety and Veterinary Office silke.bruhn@blv.admin.ch
Brunotte, Linda GERMANY	University Hospital Freiburg linda.brunotte@uniklinik-freiburg.de
Bücker, Roland GERMANY	Charité - University Medicine Berlin roland.buecker@charite.de
Budt, Matthias	Robert Koch Institute budtm@rki.de
Bulashev, Aitbay KAZAKHSTAN	Faculty of Veterinary Medicine and Livestock Technology aytbay57@mail.ru
Burger, Marica GERMANY	Robert Koch Institute buergerm@rki.de
Burger, Reinhard GERMANY	Robert Koch Institute BurgerR@rki.de
Butenschön, Frauke GERMANY	Dermatologie Universitätsklinikum fraukebutenschoen@web.de
Cadar, Daniel GERMANY	Arbovirology, Bernhard Nocht Institute for Tropical Medicine danielcadar@gmail.com

List of Participants

Name	Institute and e-mail
Chege, Stephen UK	stevmah@yahoo.com
Conraths, Franz J. GERMANY	Institut für Epidemiologie Friedrich-Loeffler-Institut franz.conraths@fli.bund.de
Daviña Nuñez, Rocio GERMANY	Influenza and Other Respiratory Viruses, Robert Koch Institute rociodavinha@outlook.com
Davoust, Bernard FRANCE	Marseille Université bernard.davoust@gmail.com
de Buhr, Nicole GERMANY	Stiftung Tierärztliche Hochschule Hannover, Institut für Mikrobiologie ndebuhr@tiho-hannover.de
de Mendonça Campos, Renata BRAZIL	Federal University of Rio De Janeiro renatacampos@micro.ufrj.br
Debski, Alina GERMANY	Free University Berlin alina.debski@gmx.de
Deeg, Katja GERMANY	Federal Office for Agriculture and Food (BLE) katja.deeg@ble.de
Demminger, Daniel GERMANY	demmingerd@rki.de
Derdakova, Marketa SLOVAKIA	Institute of Zoology, Slovak Academy of Sciences marketa.derdakova@gmail.com
Dersch, Petra GERMANY	Technische Universität Braunschweig p.dersch@tu-bs.de

List of Participants

Name	Institute and e-mail
Desiato, Rosanna ITALY	Istituto Zooprofilattico Sperimentale PLVA rosanna.desiato@izsto.it
Didyk, Yuliya UKRAINE	Acarology, Schmalhausen Institute of Zoology didykj@izan.kiev.ua
Diederich, Sandra GERMANY	Friedrich-Loeffler-Institute sandra.diederich@fli.bund.de
Dohmann, Karen GERMANY	IVD GmbH dohmann@ivd-gmbh.de
Donoghue, Dan USA	University of Arkansas ddonogh@uark.edu
Drees, Anna GERMANY	TiHo Hannover anna.drees@tiho-hannover.de
Drees, Nathalie GERMANY	Lehrstuhl für Lebensmittelsicherheit, München nathalie.drees@lmu.de
Dudek, Sabine Eva GERMANY	Westfälische Wilhelms- University of Münster Sabine.Dudek@uni-muenster.de
Dutta, Devarati INDIA	Peerless Hospital & B. K. Roy Research Centre durba_dutta_82@hotmail.com
Dzhioev, Yuri RUSSIA	State Medical University of Russian Ministry of Heath alanir07@mail.ru
Eckerle, Isabella GERMANY	University of Bonn Medical Centre eckerle@virology-bonn.de

List of Participants

Name	Institute and e-mail
Ehlers, Bernhard GERMANY	Robert Koch Institute ehlersb@rki.de
Ehrhardt, Christina GERMANY	Westfälische Wilhelms University ehrhardc@uni-muenster.de
Eibach, Daniel GERMANY	Bernhard Nocht Institute for Tropical Medicine eibach@bnitm.de
Eichhorn, Inga GERMANY	Institute of Microbiology and Epizootics Free University Berlin Centre for Infection Medicine inga.eichhorn@fu-berlin.de
Eiden, Martin GERMANY	Friedrich-Loeffler-Institut Institute for Novel and Emerging Infectious Diseases martin.eiden@fli.bund.de
Enkirch, Theresa GERMANY	Paul-Ehrlich-Institute Theresa.Enkirch@pei.de
Escalera, Marina GERMANY	Leibniz Institut Für Zoo Und Wildtierforschung Im Forschungsverbund escalera@izw-berlin.de
Esen, Meral GERMANY	Institut fürTropenmedizin meral.esen@uni-tuebingen.de
Eßbauer, Sandra GERMANY	Bundeswehr Institute of Microbiology sandraessbauer@bundeswehr.org
Falgenhauer, Linda GERMANY	Institute of Medical Microbiology linda.falgenhauer@mikrobio.med.uni-giessen.de

Name	Institute and e-mail
Feldmann, Heinz USA	National Institute of Allergy & Infectious Diseases/Rocky Mountain Laboratories feldmannh@niaid.nih.gov
Fell, Shari GERMANY	LMU München, Shari.Fell@micro.vetmed.unimuenchen.de
Ferraguti, Martina SPAIN	Estación Biológica de Doñana mferraguti@ebd.csic.es
Feßler, Andrea T. GERMANY	Friedrich-Loeffler-Institut andrea.fessler@fli.bund.de
Figuerola Borras, Jordi SPAIN	Estación Biológica de Doñana jordi@ebd.csic.es
Fischer, Sebastian GERMANY	IVD GmbH fischer@ivd-gmbh.de
Fischer, Stefan GERMANY	Friedrich-Loeffler-Institut stefan.fischer@fli.bund.de
Fleischmann, Susanne GERMANY	Institute of Microbiology and Epizootics Susanne.fleischmann@fu-berlin.de
Fonville, Judith M. UK	University of Cambridge jmf77@cam.ac.uk
Francese, Danila Raffaella ITALY	Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta VAT danilaraffaella.francese@izsto.it
Franke, Annika GERMANY	Friedrich-Loeffler-Institut bernd.hoffmann@fli.bund.de

Name	Institute and e-mail
Freidl, Gudrun THE NETHERLANDS	National Institute for Public Health and the Environment gudrun.freidl@rivm.nl
Freuling, Conrad GERMANY	Friedrich-Loeffler-Institut Conrad.Freuling@fli.bund.de
Frey, Stefan GERMANY	Bundeswehr Institute of Microbiology stefan1frey@bundeswehr.org
Friese, Anika GERMANY	Free University Berlin, Institute for Animal Hygiene and Environmental Health anika.friese@fu-berlin.de
Gan, Li Lin GERMANY	German Primate Center GmbH lgan@dpz.eu
Ghosh, Hiren GERMANY	Justus Liebig University Giessen hiren.ghosh@mikrobio.med.uni-giessen.de
Gilani, Syed-Mashkoor-Mohsin PAKISTAN	Institute of Microbiology mashkoormohsin@uaf.edu.pk
Gillespie, Thomas R. USA	Global Health Institute, Emory University gillespie@emory.edu
Goethe, Ralph GERMANY	Institut für Mikrobiologie, TiHo-Hannover ralph.goethe@tiho-hannover.de
Grobbel, Mirjam GERMANY	Federal Institute for Risk Assessment mirjam.grobbel@bfr.bund.de
Groschup, Martin GERMANY	Friedrich-Loeffler-Institut martin.groschup@fli.bund.de

Name	Institute and e-mail
Groseth, Allison USA	National Institute of Allergy and Infectious Diseases, National Institutes of Health allison.groseth@nih.gov
Grüning, Petra GERMANY	Institut für Mikrobiologie petra.gruening@tiho-hannover.de
Grützmacher, Kim GERMANY	Robert Koch Institute gruetzmacherk@rki.de
Guenther, Sebastian GERMANY	Free University Berlin sebastian.guenther@fu-berlin.de
Hacker, Jörg GERMANY	The German Academy of Sciences Leopoldina sabine.kettenmann@leopoldina.org
Haddock, Elaine CANADA	The National Institutes of Health Elaine.Haddock@nih.gov
Haklová, Božena SLOVAKIA	Institute of Parasitology SAS haklova@saske.sk
Hamm, Katharina GERMANY	Friedrich-Loeffler-Institut katharina.hamm@fli.bund.de
Hansbauer, Eva-Maria GERMANY	Robert Koch Institute hansbauere@rki.de
Hardwidge, Philip USA	Kansas State University hardwidg@gmail.com
Hauser, Elisabeth GERMANY	Universität Hohenheim elisabeth.hauser@uni-hohenheim.de
Hawkins, Elaine UK	elainehawkins88@gmail.com

Name	Institute and e-mail
Heikkinen, Petra FINLAND	Finnish Food Safety Authority Evira petra.heikkinen@evira.fi
Heimesaat, Markus M. GERMANY	Department for Microbiology and Hygiene Charité University Medicine Berlin markus.heimesaat@charite.de
Heinemann, Patrick GERMANY	Institute of Virology Charité Medical School patrick.heinemann@charite.de
Henttonen, Heikki FINLAND	Finnish Forest Research Institute Heikki.Henttonen@metla.fi
Herden, Christiane GERMANY	Justus Liebig University Institute of Veterinary Pathology christiane.herden@vetmed.uni-giessen.de
Heuer, Lea GERMANY	University of Veterinary Medicine Hannover leaheuer@gmx.net
Heuner, Klaus GERMANY	Robert Koch Institute heunerk@rki.de
Hillarius, Kirstin GERMANY	Friedrich-Loeffler-Institut kirstin.hillarius@fli.bund.de
Hille, Katja GERMANY	University of Veterinary Medicine katja.hille@tiho-hannover.de
Hoffmann, Constanze GERMANY	Robert Koch Institute HoffmannC@rki.de
Hoffmann, Markus GERMANY	University of Veterinary Medicine mhoffmann.bio@gmail.com

Name	Institute and e-mail
Hofreuter, Dirk GERMANY	Hannover Medical School; Institute for Medical Microbiology and Hospital Epidemiology hofreuter.dirk@mh-hannover.de
Hoppe, Eileen GERMANY	Robert Koch Institute HoppeE@rki.de
Hosseini, Farzaneh IRAN	University of Shahrekord hosseinifm@gmail.com
Hosseininejad, Morteza IRAN	University of Shahrekord hosseininejad@gmail.com
Hsueh, Pei-Chun TAIWAN	cat0429@gmail.com
Hurníková, Zuzana SLOVAKIA	University of Veterinary Medicine and Pharmacy in Košice hurnikova@uvlf.sk
Jäckel, Susanne GERMANY	susanne.jaeckel84@web.de
Jaeger, Timo GERMANY	German Center for Infection Research (DZIF) timo.jaeger@dzif.de
Jaeschke, Anja GERMANY	University of Bayreuth anja.jaeschke@uni-bayreuth.de
Jahfari, Setareh THE NETHERLANDS	National Institute for Public Health and Environment (RIVM) seta.jahfari@rivm.nl
Janowetz, Britta GERMANY	Bavarian Animal Health Service stephanie.bungart@tgd-bayern.de
Jansen, Friederike GERMANY	German Research Platform for Zoonoses, c/o University Muenster friederike.jansen@ukmuenster.de

Name	Institute and e-mail
Jansen, Stephanie GERMANY	Bernhard Nocht Institute for Tropical Medicine jansen@bni-hamburg.de
Janßen, Sabrina GERMANY	Medizinische Klinik Für Gastroenterologie Und Infektiologie Charité, Cbf sabrina.janssen@charite.de
Janßen, Traute GERMANY	FU Berlin, Institut für Mikrobiologie und Tierseuchen traute.janssen@fu-berlin.de
Jarvis, Michael A. UK	University of Plymouth michael.jarvis@plymouth.ac.uk
Jerome, Hanna GERMANY	Bernhard Nocht Institute for Tropical Medicine je.hanna@web.de
Johne, Reimar GERMANY	Federal Institute for Risk Assessment Reimar.Johne@bfr.bund.de
Josenhans, Christine GERMANY	Hannover Medical School josenhans.christine@mh-hannover.de
Juhls, Christiane GERMANY	Mologen AG juhls@mologen.com
Junglen, Sandra GERMANY	University of Bonn Medical Center junglen@virology-bonn.de
Junker, Barbara GERMANY	PT-DLR barbara.junker@dlr.de
Kaboutari Katadj, Jahangir IRAN	Shahrekord University kaboutari-j@vet.sku.ac.ir
Karpiskova, Renata CZECH REPUBLIC	Veterinary research Institute karpiskova@vri.cz

Name	Institute and e-mail
Kartsev, Nikolay RUSSIA	Federal Service for the Oversight of Consumer Protection and Welfare State Research Center for Applied Microbiology and Biotechnology karcev.nikolay@mail.ru
Käsbohrer, Annemarie GERMANY	Federal Institute for Risk Assessment annemarie.kaesbohrer@bfr.bund.de
Kaspers, Bernd GERMANY	University of Munich kaspers@lmu.de
Katsafadou, Angeliki GREECE	University of Thessaly agkatsaf@vet.uth.gr
Kaufmann, Eva GERMANY	Paul-Ehrlich-Institut eva.kaufmann@pei.de
Kerzhner, Alexandra GERMANY	Fraunhofer IZI alexandra.kerzhner@izi.fraunhofer.de
Klein, Joachim GERMANY	Federal Ministry of Education and Research
Klemm, Carolin GERMANY	Westfälische Wilhelms University Institute of Molecular Virology klemmc@uni-muenster.de
Klempa, Boris GERMANY	Institute of Virology Charité Medical School boris.klempa@charite.de
Klenner, Jeanette GERMANY	Centre for Biological Threats and Special Pathogens, Zbs 1, Highly Pathogenic Viruses, Robert Koch Institute klennerj@rki.de
Klewer, Kristine FRANCE	ID Vet kristine.klewer@id-vet.com

Name	Institute and e-mail
Kley, Nils	German Research Platform for Zoonoses, c/o Friedrich-Loeffler-Institut nils.kley@fli.bund.de
Klier, Christiane GERMANY	christiane-klier@t-online.de
Klotz, Christian GERMANY	Robert Koch Institute klotzc@rki.de
Koch, Sophia GERMANY	Robert Koch Institute kochs@rki.de
Koeck, Robin GERMANY	University Hospital Muenster Institute of Hygiene Robert Koch robin.koeck@ukmuenster.de
Kohl, Claudia GERMANY	Robert Koch Institute kohlc@rki.de
Köhl, Wiebke GERMANY	Bio Doc wiebke.koehl@gmx.de
Kolackova, Ivana CZECH REPUBLIC	Veterinary Research Institute kolackova@vri.cz
Kömpf, Daniela GERMANY	Landesgesundheitsamt Baden Württemberg Konsiliarlabor für Q Fieber Daniela.Koempf@rps.bwl.de
Koopmanns, Marion THE NETHERLANDS	The Erasmus University in Rotterdam m.koopmans@erasmusmc.nl
Kopp, Anne GERMANY	University of Bonn Medical Centre kopp@virology-bonn.de
Kopp, Ursula GERMANY	Projektträger im DLR ursula.kopp@dlr.de
Korup, Sarah GERMANY	Robert Koch Institute korups@rki.de

Name	Institute and e-mail
Kozlova, Irina RUSSIA	FSBI "Scientificcenter for family health and human reproduction problems" SB RAMS diwerhoz@rambler.ru
Krautkramer, Ellen GERMANY	University of Heidelberg ellen.krautkraemer@med.uni-heidelberg.de
Kreienbrock, Lothar GERMANY	Stiftung Tierärztliche Hochschule Hannover lothar.kreienbrock@tiho-hannover.de
Krüger, Maren GERMANY	Robert Koch Institute kruegerm@rki.de
Krüger, Nadine GERMANY	University of Veterinary Medicine nadine.krueger@tiho-hannover.de
Kruska, Lothar GERMANY	Pcr Labor, Mvz Dr. Stein und Kollegen lkruska@labor-stein.de
Kuhls, Katrin GERMANY	Technische Hochschule Wildau Katrin.Kuhls@th-wildau.de
Kümmerer, Beate Mareike GERMANY	University of Bonn Medical Centre kuemmerer@virology-bonn.de
Kurilshikov, Alexander RUSSIA	Institute of Chemical Biology and Fundamental Medicine SB RAS alexa.kur@gmail.com
Kurth, Andreas GERMANY	Robert Koch Institute kurtha@rki.de
Kurzrock, Lina GERMANY	University of Hohenheim lina.kurzrock@uni-hohenheim.de

List of Participants

Name	Institute and e-mail
Leendertz, Fabian GERMANY	Robert Koch Institute leendertzf@rki.de
Leggewie , Mayke GERMANY	Bernhard Nocht Institute for Tropical Medicine Leggewie@bnitm.de
Lehmann, Maria GERMANY	Bernhard Nocht Institute for Tropical Medicine maria.lehmann@bni-hamburg.de
Leno, Eugenia GERMANY	Hannover Medical School leno.eugenia@mh-hannover.de
Li, Taiyun GERMANY	Department of Veterinary Medicine the Free University Berlin Institute for Animal Hygiene and Environmental Health litaiyun606@163.com
Liedmann, Swantje GERMANY	University of Muenster s.liedmann@uni-muenster.de
Llorente, Francisco SPAIN	Centro de Investigación den SPAIN Sanidad Animal(INIA-CISA) dgracia@inia.es
Lorenz, Renate GERMANY	Mol. Virologie dr.renate.lorenz@arcor.de
Lübke-Becker, Antina GERMANY	Institut für Mikrobiologie und Tierseuchen antina.luebke-becker@fu-berlin.de
Lüdtke, Anja GERMANY	Heinrich Pette Institute anja.luedtke@hpi.uni-hamburg.de
Ludwig, Stephan GERMANY	University Muenster ludwigs@uni-muenster.de

List of Participants

Name	Institute and e-mail
Lührmann, Anja GERMANY	Universitätsklinikum Erlangen, Mikrobiologisches Institut anja.luehrmann@uk-erlangen.de
Lüschow, Dörte GERMANY	Institut für Geflügelkrankheiten, Free University Berlin doerte.lueschow@fu-berlin.de
Lynch, Phenelope UK	University of Exeter pennymath@lynch-fm.demon.co.uk
Ma, Wenjun USA	Kansas State University wjma@ksu.edu
Malkwitz, Irene GERMANY	Landeslabor Berlin-Brandenburg Irene.Malkwitz@Landeslabor-bbb.de
Marcantonio, Matteo ITALY	Department of Biodiversity and Molecular Ecology Fondazione Edmund Mach matteo.marcantonio@fmach.it
Marie, Jean-Lou FRANCE	jean-lou.marie@wanadoo.fr
Marklewitz, Marco GERMANY	Institute of Virology, University of Bonn Medical Center marklewitz@virology-bonn.de
Martellaro, Cynthia USA	Dmt, Rml/niaid/nih Hamilton cynthia.martellaro@nih.gov
Martínez-de la Puente, Josuo SPAIN	Estación Biologica de Doñana (EBD-CSIC) jmp@ebd.csic.es
Mayer, Andreas GERMANY	Bundeswehr Institute of Microbiology sandraessbauer@bundeswehr.org

Name	Institute and e-mail
Meng, Fandan GERMANY	Ti-Ho Hannover mengfandan11111@hotmail.com
Menge, Christian GERMANY	Friedrich-Loeffler-Institut christian.menge@fli.bund.de
Merle, Roswitha GERMANY	FU Berlin, Institut für Veterinär-Epidemiologie und Biometrie roswitha.merle@fu-berlin.de
Michael-Brenner, Geovana GERMANY	Institute of Farm Animal Genetics Friedrich-Loeffler-Institut (FLI) geovana.michaelbrenner@fli.bund.de
Möbius, Petra GERMANY	Friedrich-Loeffler-Institut petra.moebius@fli.bund.de
Mohamed, Yosra GERMANY	Free University Berlin Yosra.Mohamed@fu-berlin.de
Mondon, Maiken GERMANY	maiken@maxmondon.de
Moshtaghi Boroujeni, Hamdollah IRAN	Shahrekord University hmoshtaghi@yahoo.com
Mostafa, Ahmed GERMANY	Justus-Liebig University Giessen, Institute of Medical Virology ahmed.m.elsayed@bio.uni-giessen.de
Müller, Alexander GERMANY	University of Heidelberg alexander.mueller@med.uni-heidelberg.de
Müller, Hermann GERMANY	hermann.mueller@vetmed.uni-leipzig.de
Müller, Marcel A. GERMANY	University of Bonn Medical Center, Institute of Virology muller@virology-bonn.de

Name	Institute and e-mail
Müller, Stefanie GERMANY	Free University Berlin s.mueller@fu-berlin.de
Müller, Thomas GERMANY	Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut thomas.mueller@fli.bund.de
Muluneh, Aemero GERMANY	LUA Sachsen, Fachgebiet 3.2 aemero.muluneh@lua.sms.sachsen.de
Munoz-Fontela, Cesar GERMANY	Heinrich Pette Institute cesar.munoz-fontela@hpi.uni-hamburg.de
Muth, Doreen GERMANY	University of Bonn Medical Centre muth@virology-bonn.de
Napierkowski, Ira GERMANY	Institute for Medical Virology, Charité ira.napierkowski@charite.de
Neteler, Markus USA	Fondazione Edmund Mach markus.neteler@fmach.it
Niedrig, Matthias GERMANY	Robert Koch Institute niedrigm@rki.de
Nikiforuk ,Aidan CANADA	Public Health Agency of Canada nikiforuk.aidan@gmail.com
Nitsche, Andreas GERMANY	Robert Koch Institute NitscheA@rki.de
Nobach, Daniel GERMANY	Institut für Veterinär-Pathologie daniel.nobach@vetmed.unigiessen.de
Nöckler, Karsten GERMANY	Federal Institute for Risk Assessment karsten.noeckler@bfr.bund.de

List of Participants

Name	Institute and e-mail
Nowak, Kathrin GERMANY	Robert Koch Institute NowakK@rki.de
Nurtsch, Melanie GERMANY	Institut für Mikrobiologie der Bundeswehr melanienurtsch@bundeswehr.org
Oehme, Rainer GERMANY	State Health Office Baden Wuerttemberg rainer.oehme@rps.bwl.de
Oestereich, Lisa GERMANY	Bernhard Nocht Institute for Tropical Medicine oestereich@bni-hamburg.de
Olm, Sven GERMANY	MQD M-V mbH dr.olm@mqd.de
Ondrejkova, Anna SLOVAKIA	University of Veterinary Medicine and Pharmacy in Košice anna.ondrejkova@uvlf.sk
Osterrieder, Klaus GERMANY	Virologie, FU Berlin no.34@fu-berlin.de
Pan, Ming-Jeng TAIWAN	Central Taiwan University of Science and Technology mjpan@ctust.edu.tw
Papajova, Ingrid SLOVAKIA	Institute of Parasitology papaj@saske.sk
Papies, Jan GERMANY	Institute of Virology, University of Bonn Medical Center papies@virology-bonn.de
Pappas, Christopher Jason FRANCE	Unite De Biologie Des Spirochetes Institut Pasteur christopher.pappas@mville.edu

Name	Institute and e-mail
Pauly, Maude	Robert Koch Institute paulymaude@gmx.net
Percivalle, Elena ITALY	S.C. Microbiologia e Virologia IRCCS FondazionePoliclinico San Matteo e.percivalle@smatteo.pv.it
Pfeffer, Martin GERMANY	University of Leipzig pfeffer@vetmed.uni-leipzig.de
Pieper, Laura	Free University Berlin, Institut für Veterinär-Epidemiologie und Biometrie laura.pieper@fu-berlin.de
Pietsch, Michael GERMANY	Nosocomial Pathogens and Antibiotic Resistances, Robert Koch Institute pietschmi2@rki.de
Pinho dos Reis, Vinicius GERMANY	Friedrich-Loeffler-Institut Vinicius.Reis@fli.bund.de
Pintore, Maria Domenica ITALY	Istituto Zooprofilattico Sperimentale mariadomenica.pintore@ izsto.it
Pipikova, Jana SLOVAKIA	Institute of Parasitology SAS pipikova@saske.sk
Pleschka, Stephan GERMANY	Institute of Medical Virology, University of Giessen stephan.pleschka@viro.med.uni-giessen.de
Pohanka, Miroslav CZECH REPUBLIC	University of Defence miroslav.pohanka@gmail.com
Pöhlmann, Stefan GERMANY	German Primate Center GmbH spoehlmann@dpz.eu

Name	**Institute and e-mail**
Preugschas, Hannah GERMANY	Robert Koch Institute preugschash@rki.de
Prewein, Bernhard AUSTRIA	AGES Wien bernhard.prewein@ages.at
Prohl, Annette GERMANY	Friedrich-Loeffler-Institut annette.prohl@fli.bund.de
Projahn, Michaela GERMANY	Free University Berlin Institut Für Tier Und Umwelthygiene michaela.projahn@fu-berlin.de
Puppe, Astrid Norma GERMANY	Drfz, German Rheumatism Research Centre astrid.puppe@drfz.de
Rabie, Andre UK	AHVLA andre.rabie@ahvla.gsi.gov.uk
Rabold, Denise GERMANY	Ag Infektionsdiagnostik Und Molekulare Epidemiologie, Institut Für Mikrobiologie Und Tierseuchen, Freie Universität Berlin denise.rabold@fu-berlin.de
Radosa, Lukas GERMANY	Charité Berlin radosa.lukas@gmail.com
Rajasekhar, Malleshappa INDIA	drmrajasekhar@gmail.com
Ramirez de Arellano, Eva SPAIN	National Center of Microbiology eramirez@isciii.es
Rangel Vieira, Yasmin BRAZIL	Fundação Oswaldo Cruz – Fiocruz yasminerangel@ig.com.br

Name	Institute and e-mail
Reckzeh, Laura GERMANY	Robert Koch Institute reckzehl@rki.de
Redhono, Dhani INDONESIA	Sebelas Maret University dhani_ipd@yahoo.com
Reindl, Sophia GERMANY	Bernhard Nocht Institute for Tropical Medicine reindl@bni-hamburg.de
Reinhard, Kirsten GERMANY	Federal Ministry of Health
Reisberg, Kerstin GERMANY	Landesamt für Verbraucherschutz Sachsen-Anhalt kerstin.reisberg@lav.ms.sachsen-anhalt.de
Rettenbacher-Riefler, Sophie GERMANY	Niedersächsisches Landesgesundheitsamt sophie.rettenbacher-riefler@nlga.niedersachsen.de
Rettinger, Anna GERMANY	Lehrstuhl für Bakteriologie und Mykologie, Tierärztliche Fakultät, LMU München anna.rettinger@micro.vetmed.uni-muenchen.de
Richt, Jürgen A. USA	Kansas State University College of Veterinary Medicine jricht@ksu.edu
Rieger, Toni GERMANY	Bernhard Nocht Institute for Tropical Institute rieger@bni-hamburg.de
Rizzoli, Annapaola ITALY	Fondazione Edmund Mach annapaola.rizzoli@fmach.it

Name	Institute and e-mail
Roczek, Alexandra GERMANY	alexandra.roczek@lua.sms. sachsen.de
Rohde, Alexander GERMANY	Biological Safety Federal Institute for Risk Assessment alexander.rohde@bfr.bund.de
Röhrs, Susanne GERMANY	Friedrich-Loeffler-Institut susanne.roehrs@fli.bund.de
Römer, Antje GERMANY	Federal Office of Consumer Protection and Food Safety antje.roemer@bvl.bund.de
Rosà, Roberto ITALY	Fondazione Edmund Mach roberto.rosa@fmach.it
Roschanski, Nicole GERMANY	Free University Berlin nicole.roschanski@fu-berlin.de
Rossow, Marko GERMANY	Max Rubner-Institut marko.rossow@mri.bund.de
Rozycki, Miroslaw POLAND	National Veterinary Research Institute mrozycki@piwet.pulawy.pl
Rummel, Andreas GERMANY	MHH, Institut für Toxikologie rummel.andreas@mh-hannover.de
Ruppitsch, Werner AUSTRIA	Austrian Agency for Health and Food Safety werner.ruppitsch@ages.at
Sänger, Sandra GERMANY	Robert Koch Institute saengers@rki.de

Name	Institute and e-mail
Salchow, Frank GERMANY	Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit frank.salchow@laves.niedersachsen.de
Santos, Debora BRAZIL	Rural Federal University of Rio de Janeiro deborabio@gmail.com
Sas, Miriam Andrada GERMANY	Institute of Novel and Emerging Infectious Diseases Friedrich-Loeffler-Institut, Federal Institute for Animal Health MiriamAndrada.Sas@fli.bund.de
Sawatsky, Bevan GERMANY	Paul-Ehrlich-Institute bevan.sawatsky@pei.de
Schatz, Juliane GERMANY	Landeslabor Berlin-Brandenburg juliane.schatz@landeslabor-bbb.de
Schaufler, Katharina GERMANY	katharina.schaufler@fu-berlin.de
Scheinemann, Hendrik GERMANY	Gesellschaft zur Förderung von Medizin-, Bio- und Umwelttechnologien e. V. scheinemann@gmbu.de
Schlottau, Kore GERMANY	Friedrich-Loeffler-Institut kore.schlottau@fli.bund.de
Schmid, Isabell GERMANY	German Research Platform for Zoonoses, c/o University Muenster isabell.schmid@ukmuenster.de
Schmidt-Chanasit, Jonas GERMANY	Arbovirology, Bernhard Nocht Institute for Tropical Medicine jonassi@gmx.de

Name	Institute and e-mail
Schmidt, Josef SWIZERLAND	Bundesamt für Lebensmittelsicherheit und Veterinärwesen BLV josef.schmidt@bvl.admin.ch
Schmithausen, Ricarda GERMANY	University Hospital of Bonn ricarda.schmithausen@ukb.uni-bonn.de
Schräder, Tobias GERMANY	Westfaelische Wilhelms University tobias.schraeder@uni-muenster.de
Schroeder, Carsten GERMANY	QIAGEN - Sample & Assay Technologies Carsten.Schroeder@qiagen.com
Schuenadel, Livia GERMANY	Robert Koch Institute schuenadell@rki.de
Schwarz, Stefan GERMANY	Friedrich-Loeffler-Institut stefan.schwarz@fli.bund.de
Schwarzer, Sandra GERMANY	LMU München, Veterinärmedizinisches Departement, Lehrstuhl für Bakteriologie und Mykologie Sandra.Schwarzer@micro.vetmed.uni-muenchen.de
Schwemmle, Martin GERMANY	University Clinic Freiburg martin.schwemmle@uniklinik-freiburg.de
Sebastian, Franziska GERMANY	Institut für Veterinär-Pathologie franziska.sebastian@vetmed.uni-leipzig.de
Seeber, Frank GERMANY	Robert Koch Institute seeberf@rki.de
Seef, Sarah GERMANY	LDZ Aachen sseef@ukaachen.de

Name	Institute and e-mail
Segura-Caro, Juan COLOMBIA	Institución Universitaria Colegio Mayor de Antioquia juan.segura@colmayor.edu.co
Semler, Sebastian GERMANY	Technology, Methods and Infrastructure for Networked Medical Research Sebastian.Semler@tmf-ev.de
Semmler, Ilia GERMANY	German Research Platform for Zoonoses, C/O TMF e.V ilia.semmler@tmf-ev.de
Semmler, Torsten GERMANY	Freie University Berlin GERMANY Institute of Microbiology and Epizootics torsten.semmler@fu-berlin.de
Serikova, Shynar KAZAKHSTAN	Seifullin Kazakh Agro-Technical University shynara.serikova@mail.ru
Setiawaty, Vivi INDONESIA	National Institute of Health Research and Development vivisetiawaty@hotmail.com
Seyboldt, Christian GERMANY	Friedrich-Loeffler-Institut christian.seyboldt@fli.bund.de
Sharp, Hannah GERMANY	Federal Institute for Risk Assessment Hannah.Sharp@bfr.bund.de
Sheikh Ali, Hanan GERMANY	Friedrich-Loeffler-Institut sheikh.hanan@gmail.com
Shin, Dai-Lun GERMANY	Helmholtz-Zentrum für GERMANY Infektionsforschung GmbH dls12@helmholtz-hzi.de

Name	Institute and e-mail
Siew, Chern SINGAPORE	schernch@dso.org.sg
Sindern, Petra GERMANY	Bundesverband Praktizierender Tierärzte e.V. sindern@freenet.de
Singh, Vikash GERMANY	Free University Vikash.singh@fu-berlin.de
Skiba, Martin GERMANY	Robert Koch Institut SkibaM@rki.de
Sobotta, Katharina GERMANY	Friedrich-Loeffler-Institut Katharina.Sobotta@fli.bund.de
Söderholm, Sandra SWEDEN	Public Health Agency of Sweden sandra.soderholm@folkhalsomyndigheten.se
Speck, Stephanie GERMANY	Faculty of Veterinary Medicine Institute of Animal Hygiene and Veterinary Public Health stephanie.speck@vetmed.uni-leipzig.de
Splett, Kerstin GERMANY	German Research Platform for Zoonoses, c/o TMF e.V. kerstin.splett@tmf-ev.de
Stalb, Sarah GERMANY	Friedrich-Loeffler-Institut/Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis Sarah.Stalb@fli.bund.de
Stamm, Ivonne GERMANY	Microbiology Vet Med Labor GmbH Division of Idexx Laboratories ivonne-stamm@idexx.com

List of Participants

Name	Institute and e-mail
Stark, Klaus GERMANY	Robert Koch Institute starkk@rki.de
Starke, Mandy GERMANY	Bundeswehr Institute of Microbiology mandystarke@bundeswehr.org
Stech, Juergen GERMANY	Friedrich-Loeffler-Institut juergen.stech@fli.bund.de
Steinacker, Ulrike GERMANY	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit ulrike.steinacker@bvl.bund.de
Straßl, Katharina GERMANY	FU Berlin, Institute of Microbiology and Epizootics katharina.strassl@fu-berlin.de
Strauch, Eckhard GERMANY	Federal Institute for Risk Assessment eckhard.strauch@bfr.bund.de
Szabo, Kathrin GERMANY	University of Veterinary Medicine kathrin.szabo@bfr.bund.de
Szatanik, Zuzanna GERMANY	Micromol GmbH z.szatanik@micromol.com
Tappe, Dennis GERMANY	Bernhard-Nocht-Institute tappe@bnitm.de
Tedin, Karsten GERMANY	Centre for Infection Medicine, Free University Berlin karsten.tedin@fu-berlin.de
Ting, Pei Jun SINGAPORE	tpeijun@dso.org.sg
Tjaden, Nils GERMANY	University of Bayreuth nils.tjaden@uni-bayreuth.de

Name	Institute and e-mail
Tkachev, Sergey RUSSIA	Institute of Chemical Biology and Fundamental Medicine SB RAS tkachev@niboch.nsc.ru
Tsokana, Constantina GREECE	University of Thessaly kotsokan@vet.uth.gr
Turebekov, Nurkeldi GERMANY	Bundeswehr Institute of Microbiology stefan1frey@bundeswehr.org
Ulbert, Sebastian GERMANY	Fraunhofer Institute for Cell Therapy Immunology IZI sebastian.ulbert@izi.fraunhofer .de
Ulrich, Rainer G. GERMANY	Friedrich–Loeffler-Institut rainer.ulrich@fli.bund.de
Valeix, Sophie UK	s.valeix@sussex.ac.uk
Valentin, Lars GERMANY	Federal Institute for Risk Assessment lars.valentin@bfr.bund.de
Valentin-Weigand, Peter GERMANY	University of Veterinary Medicine Hannover peter.valentin@tiho-hannover.de
Van der Poel, Wim H.M. THE NETHERLANDS	Central Veterinary Institute of Wageningen University and Research Centre wim.vanderpoel@wur.nl
Van Krüchten, Andre GERMANY	Institut of Molecular Virology, Westfälische Wilhelms-University of Münster andre.van.kruechten@uni-muenster.de
Vasilakis, Nikolaos USA	University of Texas Medical Branch nivasila@utmb.edu

Name	**Institute and e-mail**
Vázquez González, Ana SPAIN	Institute of Health Carlos III a.vazquez@isciii.es
Víchová, Bronislava SLOVAKIA	Institute of Parasitology SAS vichova@saske.sk
Vincze, Szilvia GERMANY	Free University Berlin, Institute of Microbiology and Epizootics szilvia.vincze@fu-berlin.de
Vogt, Hannes GERMANY	German Research Platform for Zoonoses, c/o Friedrich-Loeffler-Institut hannes.vogt@fli.bund.de
Vojkovska, Hana CZECH REPUBLIC	Veterinary Research Institute vojkovska@vri.cz
Von Blumröder, Daniela GERMANY	Federal Ministry of Food and Agriculture
Von Brunn, Albrecht GERMANY	Max-von-Pettenkofer Institut, Ludwig-Maximilians-Universität München
Von Köckritz-Blickwede, Maren GERMANY	Stiftung Tierärztliche Hochschule mkoeckbl@tiho-hannover.de
Von Messling, Veronika GERMANY	Paul-Ehrlich-Institute veronika.vonmessling@pei.de
Voß, Claudia GERMANY	German Research Platform for Zoonoses, c/o Friedrich-Loeffler-Institut claudia.voss@fli.bund.de
Wagner, Ralf GERMANY	Paul-Ehrlich-Institute Ralf.Wagner@pei.de
Walter, Stephanie GERMANY	Twincore stephanie.walter@twincore.de

Name	Institute and e-mail
Walther, Birgit GERMANY	Institut für Mikrobiologie und Tierseuchen FU Berlin birgit.walther@fu-berlin.de
Wan, Xiufeng USA	Mississippi State University wan@cvm.msstate.edu
Wareth, Gamal GERMANY	Institute of Bacterial Infections and Zoonoses, Friedrich–Loeffler-Institut, Federal Institute for Animal Health gamalwareth@hotmail.com
Wendlandt, Sarah GERMANY	Friedrich-Loeffler-Institut sarah.wendlandt@fli.bund.de
Wendt, Anna GERMANY	Biometrie, Epidemiology and Information Processing University of Veterinary Medicine Hannover awendt@tiho-hannover.de
Wibbelt, Gudrun GERMANY	Forschungsverbund Berlin e.V. wibbelt@izw-berlin.de
Wieler, Lothar H. GERMANY	Free University Berlin lothar.wieler@fu-berlin.de
Wilking, Hendrik GERMANY	Robert Koch Institute WilkingH@rki.de
Wise, Keith AUSTRALIA	Microbiology Department Wollongong Hospital keith.wise@sesiahs.health.nsw.gov.au

List of Participants

Name	Institute and e-mail
Witkowski, Peter T. GERMANY	Institute of Virology Charité Medical School peter.witkowski@charite.de
Witte, Wolfgang GERMANY	Robert Koch Institute, Wernigerode Branch wittew@rki.de
Wolff, Thorsten GERMANY	Robert Koch Institute WolffT@RKI.de
Wrensch, Florian GERMANY	German Primate Center FWrensch@dpz.eu
Wu, Nai-Huei GERMANY	Nai-Huei.Wu@tiho-hannover.de
Yang, Wei GERMANY	Institute of Virology, TiHo Hannover yangwei0727@hotmail.com
Yao, Yancheng GERMANY	Institut für medizinischeMikrobiologie yancheng.yao@mikrobio.med.uni-giessen.de
Yeraliyeva, Lyazzat KAZAKHSTAN	Kazakh National Medical University, S.D. Asfendiyarov stefanfrey@bundeswehr.org
Zahlten, Janine GERMANY	Charité - Campus Virchow-Klinikum Janine.Zahlten@charite.de
Zhelyazkova, Petya GERMANY	Institute of Hygiene University of Münster petya.zhelyazkova@ukmuenster.de

List of Participants

Name	Institute and e-mail
Ziegler, Ute GERMANY	Friedrich-Loeffler-Institut ute.ziegler@fli.bund.de
Zimmermann, Fee GERMANY	fee.zimmermann@yahoo.fr
Zirkel, Florian GERMANY	University of Bonn Medical Centre Zirkel@virology-bonn.de
Zmora, Pawel GERMANY	Infection Biology Unit, German Primate Center pzmora@dpz.eu

INDEX

Index

The organizers would like to acknowledge the following companies whose generous support has made this meeting possible.

C · E · E · Z · A · D

Center of Excellence
for Emerging and Zoonotic Animal Diseases

Boehringer
Ingelheim

INSTITUTE FOR
INFECTIOUS ANIMAL DISEASES

A Department of Homeland Security Science & Technology Center of Excellence