TULAREMIA
INTERNATIONAL
SOCIETY

Program Agenda and Abstract Book

8th International Conference on Tularemia

September 28 – October 1, 2015

Opatija, Croatia

Table of Contents

Dear Colleagues,

Welcome to the **8th International Conference on Tularemia**, which will be held September 28 - October 1 in Opatija, Croatia. The first International Conference on Tularemia was held in 1994 in Umeå, Sweden, and subsequent conferences have been organized every three years at venues located in Europe, or in the United States. The conference has a rich history of having hosted hundreds of attendees and presenters from academia and from the clinic and industry, providing an environment wherein this interdisciplinary group has interacted and established relationships that foster advances in the *Francisella* research. The International Conferences also serve as the most important means of the Tularemia International Society to fulfill its purpose to facilitate the assembly, acquaintance and association of scientists engaged in tularemia research. The driving force behind the organization of the Tularemia meetings has been the continuous need for a conference that focuses on the clinical, applied and fundamental research of *Francisella tularensis* for future development of treatments and preventive interventions, as well as diagnosis of the disease. The forthcoming 8th International Conference will keep with past traditions by maintaining an interdisciplinary program that will highlight research efforts on the pathogenesis, cell biology, genomics, proteomics, animal models, diagnostics, vaccines, therapeutics, clinical features, ecology, epidemiology and host responses to *Francisella*. In addition, the 8th International Conference on Tularemia will feature new research directions and methods for drug and vaccine development. An overreaching goal of the four day program of the conference is to provide an environment that will serve to promote collaboration among scientists doing cutting edge research regarding Tularemia.

In view of the interdisciplinary nature of the Tularemia conference, we are honored to host this meeting and hope that all attendees will have a chance to experience the beautiful location of Opatija, as well as the island of Krk and the surroundings.

On behalf of the Scientific Committee, TULISOC and Local Organizing Committee

Marina Santic, Ph.D.
Professor, Department of Microbiology
Faculty of Medicine, University of Rijeka
Rijeka, Croatia

Anders Sjöstedt, M.D.,Ph.D.
Professor, Department of Clinical Microbiology
Umeå University
Umeå, Sweden

Acknowledgements

International Organizing Committee:

Marina Santic (University of Rijeka, Croatia) Chair
Anders Sjöstedt (University of Umeå, Sweden) Co-Chair
Wayne Conlan (National Research Council, Canada)
Karen Elkins (Food and Drug Administration, USA)
Roland Grunow (Robert Koch Institute, Germany)
Thomas Henry (INSERM, France)
Thomas Kawula (UNC School of Medicine, USA)
Petra Oyston (Porton Down, UK)
Jiri Stulik (University of Defense, Czech Republic)
David Weiss (Emory University, USA)

Tularemia International Society Executive Council:

President: Siobhán Cowley (FDA,CBER/DBPAP, USA)
Secretary: Wayne Conlan (National Research Council, Canada)
Treasurer: Petra Oyston (Porton Down, UK)

Session Chairs:

Jiri Stulik,University of Defence, Czech Republic
Thomas Henry, INSERM, France
Petra Oyston, DSTL, United Kingdom
Alain Charbit, INSERM, France
Marina Santic, University of Rijeka, Croatia
Karen Elkins, CBER/FDA, USA
Catharine Bosio, NIAID/NIH, USA
Siobhán Cowley, CBER/FDA, USA
Bradley Jones, University of Iowa, USA
Anders Sjöstedt, University of Umea, Sweden
Wayne Conlan, National Research Council, Canada
Roland Grunow, Robert Koch Institute, Germany
Monique Barel, INSERM U1151, France
David Thanassi, Stony Brook University, USA
Helena Lindgren, Umeå University, Sweden
Mats Forsman, Swedish Defence Research Agency, Sweden
Miklos Gyuranecz, Institute for Veterinary Medical Research, CAR-HAS, Hungary
Jeannine Petersen, CDC, USA

Local Organizing Committee:

Marina Santic-Chair, Medical Faculty, University of Rijeka
Mateja Ozanic-Secretary, Medical Faculty, University of Rijeka
Miljenko Doric-Secretary, Medical Faculty, University of Rijeka
Valentina Marecic, Medical Faculty, University of Rijeka
Ivana Gobin, Medical Faculty, University of Rijeka
Mirna Mihelcic, Medical Faculty, University of Rijeka
Marin Bajek, Medical Faculty, University of Rijeka

Tourist Agency Da - Riva, Opatija

Venue:
Grand Hotel 4 Opatijska Cvijeta, Opatija, Croatia

We appreciate and acknowledge major financial and logistical support from:

Tularemia International Society Executive Council

US Food and Drug Administration, Center for Biological Evaluation and Research, Silver Spring, MD

University of Rijeka

Tourist Board of the Town Opatija

Ministry of Science, Education and Sports of the Republic of Croatia

Tourist Board of Kvarner County

Town of Krk

Primorje – Gorski Kotar County

Gorea plus d.o.o.

Town of Rijeka

Scan, Jeol

Olympus d.o.o.

Zaklada Hrvatske akademije znanosti i umjetnosti

8th International Conference on Tularemia, Opatija, Croatia
September 28 – October 1, 2015

Sunday, September 27		
14:00-19:00	**Arrival and Registration**	*Grand Hotel 4 Opatijska Cvijeta*
20:00	**Welcome Reception**	*Hotel Royal*
Monday, September 28		
8:00-9:00	**Breakfast**	*Grand Hotel 4 Opatijska Cvijeta*
8:00-14:00	**Registration**	*Grand Hotel 4 Opatijska Cvijeta*
8:00-9:00	**Poster Set-up for Poster Session A**	*Meeting room „Mimoza“ Grand Hotel 4 Opatijska Cvijeta*
9:00-9:30	**Opening and Welcome** Rector of University of Rijeka Dean of Medical Faculty Marina Santic, University of Rijeka, Croatia Anders Sjöstedt, University of Umeå, Sweden	*Meeting room „Orhideja“ Grand Hotel 4 Opatijska Cvijeta*
9:30-10:30	**Keynote Address** **President of TULISOC - Siobhán Cowley, CBER/FDA, USA** MAIT cells and *Francisella* infection	
10:30-11:00	**Coffee break**	*Foyer of Meeting room „Mimoza“ Grand Hotel 4 Opatijska Cvijeta*
11:00-12:50	**Session 1:** Genomics, Proteomics, and Genetics of *Francisella* **Chairs:** Jiri Stulik, University of Defence, Czech Republic Thomas Henry, INSERM, France	*Meeting room „Orhideja“ Grand Hotel 4 Opatijska Cvijeta*
11:00-11:25	**Ivo Fabrik, University of Defence, Czech Republic** Proteomic mapping of cell signaling in dendritic cells during early interactions with *Francisella tularensis*	
11:25-11:50	**Dave Wagner, Northern Arizona University, USA** New lessons from recent phylogeographic studies of *Francisella tularensis*: evidence for long-distance dispersal and a quiescent environmental phase	

11:50-12:10	**Kathryn Ramsey, Boston Childrens Hospital, USA** Ubiquitous promoter-localization of essential virulence regulators in *Francisella tularensis*	
12:10-12:30	**Klaus Heuner, Robert Koch Institute, Germany** Identification of a Genomic Island FhaGI-1 of *Francisella tularensis* subsp. *novicida*-like strain 3523 generating episomal circular forms	
12:30-12:50	**Anders Johansson, Umeå University, Sweden** Evolutionary snapshots of *Francisella tularensis* captured by genomic surveillance of outbreaks	
12:50-13:50	**Lunch**	*Grand Hotel 4 Opatijska Cvijeta*
13:50-15:45	**Session 2:** The Bacterium *Francisella*: Bacterial Physiology, Biochemistry, and Adaptation **Chairs:** Petra Oyston, DSTL, United Kingdom Alain Charbit, INSERM, France	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
13:50-14:15	**Alain Charbit, INSERM, France** The multiple ways of fueling the TCA cycle of intracellular *Francisella*	
14:15-14:40	**Jason Huntley, University of Toledo, USA** DsbA: the Achilles heel of *Francisella tularensis*	
14:40-15:05	**Petra Oyston, DSTL, United Kingdom** The stringent response in *Francisella tularensis*	
15:05-15:25	**Daniel Clemens, University of California, USA** Atomic structure and functional analysis of *Francisella* type VI secretion system	
15:25-15:45	**David Thanassi, Stony Brook University, USA** Links between carbon metabolism, oxidative stress and production of outer membrane vesicles and tubes by *Francisella novicida*	
15:45-16:15	**Coffee break**	*Foyer of Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*
16:15-17:30	**Session 3:** Short Presentations **Chairs:** Monique Barel, INSERM U1151, France David Thanassi, Stony Brook University, USA	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
16:15-16:30	**Chinmay Dwibedi, Umeå University, Sweden** On how tularemia entered and found its way through western Europe – a genomic epidemiology study	
16:30-16:45	**Kerstin Myrtennas, Swedish Defence research Agency, Sweden** Towards a global phylogeography of *Francisella tularensis* subspecies *holarctica*	

16:45-17:00	**Patricia Renesto, CNRS, France** DNA supercoiling activity of DNA gyrases from *Francisella tularensis* strains resistant to quinolones	
17:00-17:15	**Aynur Karadenizli, Kocaeli University,Turkey** Proteomic analysis of *Francisella tularensis* subspecies *holarctic*a isolates from patients and environment in tularemia epidemics	
17:15-17:30	**Thomas Inzana, Virginia Tech, USA** Loss of O-antigen and deglycosylation of the capsule-like complex promote biofilm formation in *Francisella tularensis*	
17:30-19:00	**Session 4:** **Poster Session A**	*Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*
Tuesday, September 29		
8:00-9:00	**Breakfast**	*Grand Hotel 4 Opatijska Cvijeta*
8:00-9:00	**Poster set up for Poster Session B**	*Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*
9:00-11:05	**Session 5:** Cell Biology of *Francisella* **Chairs:** Marina Santic, University of Rijeka, Croatia Helena Lindgren, Umeå University, Sweden	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
9:00-9:25	**Simon Dove, Boston Children's Hospital, USA** Control of a putative anti-virulence factor	
9:25-9:50	**Thomas Henry, INSERM, France** Guanylate-binding proteins promote *Francisella novicida* lysis in the host cytosol and Aim2 inflammasome activation	
9:50-10:05	**Athar Alam, Umeå University, Sweden** The enigmatic role of the ClpB protein of *Francisella tularensis*	
10:05-10:20	**Marina Santic, University of Rijeka, Croatia** Intracellular trafficking of *Francisella tularensis* subsp. *novicida* within *Dictyostelium discoideum*	
10:20-10:35	**Anders Sjöstedt, Umeå University, Sweden** *Francisella* and the phagosomal escape – insights to a potential role of the *Francisella* T6SS	
10:35-10:50	**Daniel Powell, University of Arizona, USA** Functional NRAMP overcomes the requirement for TLR signaling in *Francisella* infection	
10:50-11:05	**Tom Kawula, University of North Carolina, USA** *Francisella* cell to cell transfer	
11:05-12:20	**Session 6:** **Poster Session B with coffee**	*Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*

12:20-13:00	**TULISOC meeting**	*Meeting room „Orhideja“ Grand Hotel 4 Opatijska Cvijeta*
13:00-14:00	**Lunch**	*Grand Hotel 4 Opatijska Cvijeta*
15:00	Conference Excursion	
Wednesday, September 30		
8:00-9:00	**Breakfast**	*Grand Hotel 4 Opatijska Cvijeta*
8:00-9:00	**Poster Set-up for Poster Session C**	*Meeting room „Mimoza“ Grand Hotel 4 Opatijska Cvijeta*
9:00-10:50	**Session 7, Part A:** Host responses to *Francisella* **Chairs:** Karen Elkins, CBER/FDA, USA Catharine Bosio, NIAID/NIH, USA	*Meeting room „Orhideja Grand Hotel 4 Opatijska Cvijeta*
9:00-9:25	**Catherine Bosio, NIAID/NIH, USA** Non –proteinaceous virulence factors of *Francisella tularensis*	
9:25-9:50	**Bradley Jones, University of Iowa, USA** Characterizing differences between LVS strains and the implications for eliciting immunity	
9:50-10:10	**Monique Barel, INSERM, France** *Francisella* manipulates host glycosylation pathways and the UPR response in human macrophages	
10:10-10:30	**Susan Brock, University of Kansas, USA** Complement C3 as a determinant of macrophage death during *Francisella* infection	
10:30-10:50	**Bernard Arulanandam, University of Texas at San Antonio, USA** Intestinal goblet cells can augment m-cells for entry of oral vaccines	
10:50-11:10	**Coffee break**	*Foyer of Meeting room „Mimoza“ Grand Hotel 4 Opatijska Cvijeta*
11:10-13:00	**Session 7, Part B:** Host responses to *Francisella* **Chairs:** Siobhán Cowley, CBER/FDA, USA Bradley Jones, University of Iowa, USA	*Meeting room „Orhideja“ Grand Hotel 4 Opatijska Cvijeta*
11:10-11:35	**Roberto De Pascalis, CBER/FDA, USA** Correlates of protection against *Francisella tularensis:* are we there yet?	
11:35-12:00	**Wayne Conlan, National Research Council, Canada** *Francisella tularensis* virulence: Still scratching at the surface	

12:00-12:20	**Vienna Brown, Colorado State University, USA** Evaluation of pathogenesis and immune response of *Francisella tularensis* in cottontail rabbits	
12:20-12:40	**Aimee Cunningham, University of Maryland Baltimore, USA** Characterization of the live attenuated Schu S4Δ*aroD* vaccine candidate against pulmonary tularemia	
12:40-13:00	**Hanne Winther-Larsen, University of Oslo, Norway** Dissection of *Francisella* virulence in *Dictyostelium*; a genetically tractable host system	
13:00-14:00	**Lunch**	*Grand Hotel 4 Opatijska Cvijeta*
14:00-15:50	**Session 8:** Vaccines, Therapeutics and Diagnostics for Tularemia **Chairs:** Anders Sjöstedt, University of Umeå, Sweden Wayne Conlan, National Research Council, Canada	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
14:00-14:25	**Eileen Barry, University of Maryland, USA** Development of efficacious SCHU S4-based live attenuated *Francisella* vaccine strains	
14:25-14:50	**Zusana Kročová, University of Defence, Czech Republic** The role of B cells in the course of *F. tularensis* infection	
14:50-15:10	**Max Maurin, CHU Grenoble, France** Antibiotic resistance in *Francisella tularensis*: in vitro and in vivo evaluation	
15:10-15:30	**Karl Klose, University of Texas San Antonio, USA** Development of a Novel Vaccine Against Tularemia	
15:30-15:50	**Laura Marshall, Dstl, UK** Protection with a glyco-conjugate vaccine against aerosol delivered *Francisella tularensis* Schu S4 in Fischer 344 rats	
15:50-17:50	**Session 9:** **Poster Session C with coffee**	*Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*
19:00	**Gala Dinner**	*Hotel Royal*
Thursday, October 1		
8:00-9:00	**Breakfast**	*Grand Hotel 4 Opatijska Cvijeta*

9:00-10:30	**Session 10:** Short Presentations **Chairs:** Mats Forsman, Swedish Defence Research Agency, Sweden Miklos Gyuranecz, Institute for Veterinary Medical Research, CAR-HAS, Hungary	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
9:00-9:15	**Marcus Horwitz, UCLA, USA** Stimulus responsive mesoporous silica nanoparticles provide controlled release of moxifloxacin and enhanced efficacy against pneumonic tularemia in mice	
9:15-9:30	**Massimo Fabbi, Institut Zooprofilattico Sperimentale, Italy** No evidence of transovarial transmission of *Francisella tularensis* by tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*	
9:30-9:45	**Miklos Gyuranecz, Institute for Veterinary Medical Research, CAR-HAS Hungary** Detection of virulence difference between b.13 (“red”) and ftnf002-00 (“purple”) *Francisella tularensis* ssp. *holarctica* genotypes	
9:45-10:00	**Vitaly Pavlov, SRCAMB, Russia** Analysis of *Francisella tularensis* strains isolated from humans and small rodents during year 2013 tularemia outbreak in Khanty-mansiysk sity, West Siberia	
10:00-10:15	**Helena Lindgren, Umeå University, Sweden** Gallium potentiates the antibacterial effect of gentamicin agains *Francisella tularensis*	
10:15-10:30	**Tina Guina, The National Institute of Allergy and Infectious Diseases (NIAID), USA** Qualification of cynomolgus macaque model of pulmonary tularemia	
10:30-11:00	**Coffee break**	*Foyer of Meeting room „Mimoza“* *Grand Hotel 4 Opatijska Cvijeta*
11:00-12:50	**Session 11:** Clinical Disease, Epidemiology, and Ecology of Tularemia **Chairs:** Roland Grunow, Robert Koch Institute, Germany Jeannine Petersen, CDC, USA	*Meeting room „Orhideja“* *Grand Hotel 4 Opatijska Cvijeta*
11:00-11:25	**Jeannine Petersen, CDC, USA** Genomic Mutations Linked to Virulence Differences Among *Francisella tularensis* A1 Strains	
11:25-11:50	**Roland Grunow, Robert Koch Institute, Germany** The unexpected is most fascinating – tularemia in a niche of Germany revealed a new subclade of clade B.12	

11:50-12:10	**Mats Forsman, Swedish Defence Research Agency, Sweden** Erythromycin resistance in *Francisella tularensis* is explained by a single point mutation that occurred only once during the evolution of the species	
12:10-12:30	**Irina Mesheryakova, N.F. Gamaleya research Centre of Epidemiology and Microbiology, Russia** Vector-born epidemic (group disease) outbreaks of tularemia in Russian Federation in XXI century	
12:30-12:50	**Patrik Rydén, Umea University, Sweden** A novel approach for modelling the geographical risk distribution of getting tularemia and determining change over time	
13:00	**Closing** Marina Santic, Univeristy of Rijeka, Croatia Anders Sjöstedt, Univeristy of Umeå, Sweden	*Meeting room „Orhideja“ Grand Hotel 4 Opatijska Cvijeta*

Session 1:

Genomics, Proteomics, and Genetics of *Francisella*

Session 1: Genomics, Proteomics, and Genetics of *Francisella*

Chairs: Jiri Stulik
University of Defence, Czech Republic

Thomas Henry
INSERM, France

S1-1 Proteomic mapping of cell signaling in dendritic cells during early interactions with *Francisella tularensis*
Ivo Fabrik, Marek Link, Daniela Putzova, Pavel Rehulka, Jiri Stulik

S1-2 New lessons from recent phylogeographic studies of *Francisella tularensis*: evidence for long-distance dispersal and a quiescent environmental phase
David M. Wagner, Anders Johansson, Dawn Birdsell, Kerstin Myrtennäs, Johanna Thelaus, Pär Larsson, Mats Forsman, Paul Keim

S1-3 Ubiquitous promoter-localization of essential virulence regulators in *Francisella tularensis*
Kathryn M. Ramsey

S1-4 Identification of a genomic island FhaGI-1 of *Francisella tularensis* subsp. *novicida*-like strain 3523 generating episomal circular forms
Kerstin Rydzewski, Roland Grunow and Klaus Heuner

S1-5 Evolutionary snapshots of *Francisella tularensis* captured by genomic surveillance of outbreaks
Chinmay Dwibedi, Adrian Lärkeryd, Kerstin Myrtennäs, Linda Vidman, Petter Lindgren, Caroline Öhrman, Dawn Birdsell, David Wagner, Paul Keim , Patrik Ryden ,Mats Forsman, Pär Larsson, Anders Johansson

S1-1

Proteomic mapping of cell signaling in dendritic cells during early interactions with *Francisella tularensis*

Ivo Fabrik, Marek Link, Daniela Putzova, Pavel Rehulka, Jiri Stulik

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

Dendritic cells (DCs) represent potentially important host cells for *Francisella tularensis*. As professional phagocytes, DCs are susceptible to *Francisella* replication. In addition, the bacterium can utilize DC's migratory behavior and immunity-priming capabilities for its own benefits, which may ultimately shape the course of infection on the systemic level. However, to better understand consequences of the *Francisella*-DC interaction, one would need to have the detailed knowledge of the interplay on the molecular level. Methods of system biology provide valuable help in this regard as these are able to monitor the global state rather than single events. In this study, quantitative shotgun proteomics was applied to uncover changes in the cell signaling of murine bone marrow-derived DCs (BMDCs) during early contacts with *Francisella tularensis* subsp. *holarctica* FSC200 *in vitro*. The work was focused on the proteome of lipid rafts (isolated as detergent-resistant membranes) and the whole-cell phosphoproteome as these are expected to be primarily affected in BMDCs during the bacterial invasion. A modified protocol for Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) approach adapted to the *in vitro* cultivation of primary BMDCs was used for the proteome/phosphosite quantitation. Lipid raft proteome and the whole-cell phosphoproteome were isolated using sucrose-density fractionation and peptide-based hydrophilic interaction chromatography fractionation/TiO_2 enrichment, respectively. The samples were analyzed using nano-scale liquid chromatography and high-resolution mass spectrometry. In total, more than 1,200 proteins from lipid rafts and 17,000 phosphosites from the whole-cell lysate were identified in infected BMDCs. While lipid rafts proteins showed rather subtle changes, a considerable increase in the number of phosphorylation events was observed following *Francisella* invasion. Several protein kinases were predicted to participate on signaling based on the kinase motif analysis (e.g. protein kinase B). Although the notable part of DC proteins affected by the infection were involved in the regulation of cytoskeleton, vesicular trafficking or RNA processing, many other metabolic pathways and cellular events emerge from the data as activated. The identified part of signaling pathways controlling the response of DCs to *Francisella* is expected to expand with the ongoing experiments and the corresponding data interpretation.

S1-2

New lessons from recent phylogeographic studies of *Francisella tularensis*: evidence for long-distance dispersal and a quiescent environmental phase

David M. Wagner[1], Anders Johansson[2], Dawn Birdsell[1], Kerstin Myrtennäs[3], Johanna Thelaus[3], Pär Larsson[3], Mats Forsman[3], Paul Keim[1]

[1] Northern Arizona University, Flagstaff, AZ, USA; [2] Umeå University, Umeå, Sweden; [3] Swedish Defense Research Agency, Umeå, Sweden
address: Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, Arizona, USA. Dave.Wagner@nau.edu

The ecology of *Francisella tularensis* remains poorly understood, including the mechanisms by which it persists in the environment. In addition, despite extensive sampling in some locations, such as Sweden, the overall global phylogeography of *F. tularensis* is also poorly understood. There is now a global phylogenetic framework, based upon whole genome sequencing data, which allows strains from new locations to be directly compared to previously described strains from other locations. Placing new and existing strains in this framework has yielded important new insights about *F. tularensis*. For example, it now seems likely that previous suggestions that *F. tularensis* subspecies *holarctica* may have originated in Scandinavia or in the United States need to be reevaluated. These arguments were based upon the large phylogenetic diversity observed in these countries. However, new studies have revealed similar diversity in other countries, such as Turkey and China, leaving the likely origin of this subspecies in question. Indeed, across numerous recent studies extensive sampling efforts have revealed significant phylogenetic diversity and documented that numerous phylogenetic groups co-occur in most regions and are involved in human disease. The widespread nature of many of the phylogenetic groups within *F. tularensis* subspecies *holarctica* suggests that there has been long-dispersal of this subspecies, either recently or in the past. Long-distance dispersal is also apparent on smaller spatial scales, as several studies in Sweden and elsewhere in Europe have identified multiple strains that are 100% identical with whole genome sequencing data, even though they were isolated sometimes hundreds of kilometers apart in geographic space. Identical strains also can be separated by large distances in temporal space, with some identical strains isolated from the environment more than 10 years apart. This latter finding is suggestive of a quiescent phase for *F. tularensis* subspecies *holarctica* in the environment in which it does not replicate but still persists for long periods of time. New laboratory studies find similar patterns of persistence in a quiescent phase with limited to no evidence for replication.

S1-3

Ubiquitous promoter-localization of essential virulence regulators in *Francisella tularensis*

Kathryn M. Ramsey

Boston Childrens Hospital, Boston, USA

In order to cause disease, the Gram-negative pathogen *Francisella tularensis* requires the coordinate activity of three transcription regulators: MglA, SspA, and PigR. MglA and SspA form a complex that interacts with RNA polymerase (RNAP), and PigR is a putative DNA-binding protein that functions through interaction with the MglA-SspA complex. Together, MglA, SspA, and PigR have been shown to control the expression of approximately 100 genes, including those on the Francisella Pathogenicity Island (FPI). While most transcription regulators are thought to only be found at the promoters that they regulate, we show using chromatin immunoprecipitation coupled with high-throughput DNA sequencing (ChIP-Seq) that these three transcription regulators are found not only at those promoters they control, but also at essentially all promoters in *F. tularensis*. In addition, we determine that the ability of PigR to localize to promoter regions is dependent on the presence of MglA, suggesting that the interaction between PigR and the MglA-SspA complex specifically directs PigR to promoter regions. Finally, we have determined that there is a specific 7 base pair sequence element present in the promoters of regulated genes that allows PigR (and thus the MglA-SspA complex) to positively regulate gene expression. Thus, these three principal virulence regulators in *F. tularensis* function in a non-classical manner, being present at virtually all promoters while only activating transcription from a subset of promoters containing a specific sequence element.

S1-4

Identification of a genomic island fhagi-1 of *Francisella tularensis* subsp. *novicida* - like strain 3523 generating episomal circular forms

Kerstin Rydzewski, Roland Grunow and Klaus Heuner

Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany

We identified a putative prophage on a genomic island (GI) within the genome sequence of *Francisella hispaniensis* isolate AS0-814 (*F. tularensis* subsp. *novicida*-like 3523) by the analysis of spacer DNAs of the CRISPR-Cas systems of different *F. tularensis* subsp. *novicida* strains (Schunder et al., 2013; IJMM 303:51-60). Here we report the identification of the genomic island (FhaGI-1) as a mobile element which is able to form a episomal circular structure. The episomal form of FhaGI-1 is generated by *F. hispaniensis* and the excision of the island is an integrase-dependent and site-specific process. We could also show that a small variant of the island is functional and after its electroporation into strain *F. tularensis* subsp. *holarctica* LVS, the GI was stable and site-specific integrated into the genome of the transformants. The experiments revealed that the integrase is sufficient for the integration and excision of the small variants into and from the DNA backbone, respectively. Furthermore, we could demonstrate that the genomic island is also functional (episomal form formation) in other bacterial species (*Escherichia coli*). Thus the element could be used as a genetic tool in *Francisella tularensis* research, e.g. for stable complementation of specific gene-deletion mutants. Furthermore, we identified the $tRNA^{Val}$ gene of *Francisella* as an integration site for genomic islands, for example for FphGI-1 of *F. philomiragia* ATCC 25016. However, in this case we were not able to detect the episomal form of the genomic island, probably due to a mutated repeat (integration) region. Nevertheless, we can demonstrate that integrative genomic islands are present in *Francisella* and that some of these elements may allow horizontal gene transfer between different *Francisella* species.

S1-5

Evolutionary snapshots of *Francisella tularensis* captured by genomic surveillance of outbreaks

Chinmay Dwibedi[1,2], Adrian Lärkeryd[1,2], Kerstin Myrtennäs[2], Linda Vidman[1], Petter Lindgren[2], Caroline Öhrman[2], Dawn Birdsell[3], David Wagner[3], Paul Keim[3], Patrik Ryden[1],Mats Forsman[2], Pär Larsson[2], Anders Johansson[1]

[1] Umeå University, Umeå, Sweden; [2] Swedish Defense Research Agency, Umeå, Sweden; [3] Northern Arizona University, Flagstaff, AZ, USA; address: Department of clinical microbiology, Clinical bacteriology, and Laboratory for molecular infection medicine Sweden (MIMS), Umeå University, SE-901 85 Umeå, Sweden, anders.f.johansson@umu.se

Objective: Geographically restricted seasonal outbreaks of tularemia are common in several regions of Sweden. The causative bacterium, *Francisella tularensis*, is mainly transmitted to humans by arthropod vectors resulting in tularemia characterized by fever, an infected skin ulcer, and corresponding tender enlarged lymph nodes. Detailed genetic and geographical analysis of *F. tularensis* cultured from humans with disease can identify geographical sources of infection and dispersal patterns. Additionally, the evolution, replication history and other population genetic parameters of *F. tularensis* may be inferred.
Methods: We analyzed the whole genome data of *F. tularensis* isolates cultured from 138 individuals who contracted tularemia in Örebro County, Sweden from 1994 to 2010. The genomic data was further compared to a global database of 600 reference genomes and we identified 27 genomes not originating in Örebro County that differed within two single nucleotide polymorphisms (SNPs) to any genome from Örebro. The genomes were then classified into phylogenetic clades. Whole genome phylogenetic patterns were compared with geographical coordinates and time of the infection to infer disease spread and analyze population parameters. Mutation rates were estimated by Bayesian methods and by performing *in vivo* animal experiments.
Results: Several phylogenetic clades of *F. tularensis* were identified across seven outbreaks in Örebro County with very little variation within these clades. Identical clones (100% SNP identity) were observed to have persisted for years. The clades showed different patterns of evolution, suggesting some of them were locally evolved in Örebro County whereas others had close genetic neighbors at distant places in Sweden consistent with rapid long distance dispersal. Based on the genomic data, we could retrospectively pin-point the place of contraction of tularemia for two humans that only temporarily visited Örebro and had incorrect spatial data for disease contraction in the database. The mutation rate analysis of *F. tularensis* from humans indicated null rates.
Conclusion: The genomic diversity among the strains confirmed several subpopulations of *F. tularensis* were simultaneously present in each outbreak.

Persistence of multiple clones for several years with no spatial or temporal correlation was observed, indicating extremely low mutation and replication rates. Evolutionary snapshots were captured including the depiction of clone elimination in recent outbreaks, a phenomenon which may result from the purging of new slightly deleterious mutations by natural selection. The outbreak investigation also demonstrated that, with an extensive genome sequence database, clone tracking of *F. tularensis* is possible solely by genomic analysis without using spatial data.

Session 2:

The Bacterium *Francisella*: Bacterial Physiology, Biochemistry and Adaptation

Session 2: The Bacterium *Francisella*: Bacterial Physiology, Biochemistry, and Adaptation

Chairs: Petra Oyston
DSTL, United Kingdom

Alain Charbit
INSERM, France

S2-1 The multiple ways of fueling the TCA cycle of intracellular *Francisella*
Terry Brissac, Jason Ziveri, Elodie Ramond, Gael Gesbert, Fabiola Tros, Marion Dupuis, Monique Barel, Edern Cahoreau, Alain Charbit

S2-2 DsbA: the Achilles heel of *Francisella tularensis*
Jason F. Huntley

S2-3 The stringent response in *Francisella tularensis*
Amber L. Murch, Peter L. Roach, Petra C. F. Oyston

S2-4 Atomic structure and functional analysis of *Francisella* type VI secretion system
Daniel L. Clemens, Peng Ge, Bai-Yu Lee, Hong Zhou, and Marcus Horwitz

S2-5 Links between carbon metabolism, oxidative stress and production of outer membrane vesicles and tubes by *Francisella novicida*
Vinaya Sampath and David G. Thanassi

S2-1

The multiple ways of fueling the TCA cycle of intracellular *Francisella*

Terry Brissac[1,2], Jason Ziveri[1,2], Elodie Ramond[1,2], Gael Gesbert[1,2], Fabiola Tros[1,2], Marion Dupuis[1,2], Monique Barel[1,2], Edern Cahoreau[3,4,5], Alain Charbit[1,2]

[1] Université Paris Descartes, Sorbonne Paris Cité, Bâtiment Leriche, Paris, France; [2] INSERM U1151 - CNRS UMR 8253, Institut Necker-Enfants Malades. Equipe 11: Pathogénie des Infections Systémiques, Paris, France; [3] Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, 31077 Toulouse, France; [4] INRA, UMR792, Ingénierie des Systèmes Biologiques et des Procédés, 31400 Toulouse, France; [5] CNRS, UMR5504, 31400 Toulouse, France

Francisella tularensis is able to infect a variety of cell types but replicates in mammalian hosts mainly in the cytosol of infected macrophages. We have recently shown that *Francisella* relied on multiple host-derived amino acids as carbon and energy sources during its intracellular life cycle. Our current studies demonstrate that gluconeogenesis constitutes a major metabolic pathway in *Francisella* pathogenesis. In particular, inactivation of the gene responsible for the conversion of fructose1,6-bisphosphate to fructose 6-phosphate impaired cytosolic multiplication of the pathogen, under limiting glucose availability and severely affects virulence in the mouse model. Isotopic profiling revealed the major role of the Embden-Meyerhof (glycolysis) pathway in glucose catabolism in *Francisella*. Importantly, amino acids appear to be major contributors to fuel this pathway, confirming links between amino acid uptake and central metabolic pathways.

S2-2

DsbA: the achilles heel of gram-negative pathogens

Jason F. Huntley

Department of Medical Microbiology and Immunology, University of Toledo, Toledo, Ohio, U.S.A.

Bacterial pathogens require efficient systems to quickly form and maintain correct disulfide bonds for protein stability and function. This is especially true for intracellular pathogens, which are exposed to toxic molecules such as superoxide and nitric oxide. The intracellular pathogen *Francisella tularensis* encodes a disulfide bond formation protein ortholog, DsbA, which previously was reported to be required for infection of macrophages and mice. However, the molecular mechanisms by which *F. tularensis* DsbA contributes to virulence were unknown. In recent studies, we have demonstrated that *F. tularensis* DsbA is a bifunctional protein that oxidizes and, more importantly, isomerizes complex disulfide connectivity in substrates. A single amino acid in the conserved *cis*-proline loop of the DsbA thioredoxin domain was shown to modulate both isomerase activity and *F. tularensis* virulence. Using a molecular trapping approach, we identified over 50 DsbA substrates in *F. tularensis*, including outer membrane proteins, virulence factors, and many hypothetical proteins. The majority of these substrates contain three or more cysteines, indicating that they likely require DsbA isomerase activity to form correct disulfide bond linkages. Current efforts are dissecting the molecular mechanisms by which these DsbA substrates, particularly the hypothetical proteins, are required for *F. tularensis* virulence. One DsbA substrate, FTT_0509c, is required for *F. tularensis* virulence as knockouts in the Type A strain SchuS4 are completely attenuated. We propose that the extreme virulence of *F. tularensis* is partially due to the bifunctional nature of DsbA, that many of the newly-identified DsbA substrates are required for virulence, and that the development of future DsbA inhibitors could have broad anti-bacterial implications.

S2-3

The stringent response in *Francisella tularensis*

Amber L. Murch[1], Peter L. Roach[2], Petra C. F. Oyston[1]

[1] Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ; [2] School of Chemistry, Faculty of Natural & Environmental Sciences, University of Southampton SO17 1BJ, UK

Under conditions of nutrient limitation, bacteria initiate the stringent response, co-ordinated by the signalling nucleotides guanosine tetra- and penta-phosphate, collectively termed (p)ppGpp. During starvation, (p)ppGpp accumulates and coordinates diverse transcriptional alterations. (p)ppGpp levels are controlled by two enzymes, RelA and SpoT, which synthesise (p)ppGpp. Inorganic polyphosphate, a global regulatory molecule, has also been linked to the stringent response. Levels of polyphosphate are controlled by the enzymes polyphosphate kinase (PpK) and exopolyphosphatase (PpX). Mutation of *relA* and *spoT* results not only in abrogation of (p)ppGpp production, but also results in lower levels of polyphosphate accumulation. However, the interaction of the stringent response with the polyphosphate regulon is not clearly understood. Previously, we reported that mutants of *F. tularensis* defective in production of polyphosphate and (p)ppGpp exhibited defects for intracellular growth in macrophages and were attenuated in mice. We report here analysis of the stringent regulon, triggered using serine hydroxymate, which consisted of 1089 genes, 65.80 % of *F. tularensis* SCHU S4 total annotated open reading frames. Of these, 316 genes were differentially regulated more than two-fold. Titration of serine hydroxamate revealed the stringent response to be an on/off response, with little change in the profile with increasing concentration. As expected, genes involved with protein synthesis and energy metabolism were strongly affected in expression, but known virulence factors were also affected. The largest number of differentially regulated genes grouped by functional category were genes of unknown function. Work is ongoing to validate the results, and elucidate the contribution of selected genes to the *Francisella* stringent response.

S2-4

Atomic structure and functional analysis of *Francisella* type VI secretion sysyem

Daniel L. Clemens[1], Peng Ge[2,3], Bai-Yu Lee[1], Hong Zhou[2,3] and Marcus Horwitz[1,2]

[1] Division of Infectious Diseases, Department of Medicine, School of Medicine, University of California–Los Angeles; [2] Department of Microbiology, Immunology and Molecular Genetics, School of Medicine, University of California–Los Angeles; [3] The California NanoSystems Institute (CNSI), University of California–Los Angeles; Department of Medicine, CHS 37-121, School of Medicine, University of California–Los Angeles, 10833 Le Conte Avenue, Los Angeles, California 90095-1688,USA

Type VI secretion systems (T6SSs) are newly identified contractile nanomachines in Gram-negative bacteria that translocate effector proteins across bacterial membranes into eukaryotic or prokaryotic target cells. The *Francisella* pathogenicity island (FPI) is required for bacterial phagosome escape, intracellular replication and virulence. Based on limited homologies to T6SSs of other bacteria, it has been assumed that FPI encodes a T6SS-like apparatus, but this has also been challenged because of the limited degree of homology and the apparent absence of other key components present in other T6SSs. We employed split GFP-technology to demonstrate that two FPI proteins, IglA and IglB, interact to form fluorescent macromolecular structures (fluorescent foci) in response to specific environmental conditions. We identified high KCl and the macrophage environment as conditions that trigger formation of the fluorescent macromolecular structures, and we confirmed that assembly of the structure is associated with secretion of two putative effector proteins, VgrG and IglC. We purified the IglA/B macromolecular structures from *F. novicida* and used state-of-the-art cryo electron microscopy with direct electron detection and helical reconstruction to determine the 3-D structure of the *Francisella* T6SS post-contraction sheath at 3.7Å and built an atomic model of the apparatus. Our data demonstrate that the *Francisella* T6SS sheath structure is organized as a cylindrical helix with an axial six-fold rotational symmetry that has structural homology to bacterial R2 pyocins and to contractile phages such as T4. Our atomic model of the *F. novicida* T6SS outer sheath is likely to apply also to the *F. tularensis* subsp. *tularensis* T6SS outer sheath, because IglA and IglB of these two species show 98% and 100% identity, respectively. The *Francisella novicida* T6SS sheath is constructed with discs of six heterodimers of IglA/IglB proteins that interlace into a two-dimensional mesh with augmented β sheets contributed by the N-terminus of IglA and the C-terminus of IglB. By structure-based mutagenesis, using mutants with deletions of the N-terminus of IglA or the C-terminus of IglB, we demonstrated that the integrity of this two-dimensional mesh architecture is crucial to *Francisella* secretion, phagosomal escape, and intracellular replication. Our findings will facilitate design of drugs targeting this highly prevalent secretion apparatus, which is pivotal to the virulence of many pathogenic Gram-negative bacteria.

S2-5

Links between carbon metabolism, oxidative stress and production of outer membrane vesicles and tubes by *Francisella novicida*

Vinaya Sampath and David G. Thanassi

Department of Molecular Genetics and Microbiology, Stony Brook University Center for Infectious Diseases, Stony Brook University, Stony Brook, NY 11794-5120, USA

Many Gram-negative pathogenic bacteria secrete outer membrane vesicles (OMV) as a virulence strategy to influence their host environment. However, little is known about the signals and mechanisms regulating production of OMV by bacteria. We have previously defined growth phase and culture conditions under which *Francisella novicida* secretes typical, spherical OMV, along with novel, tube-shaped vesicles that are derived from the outer membrane. Production of the *F. novicida* tubes is stimulated in the presence of macrophages, and mice vaccinated with OMV and tubes are protected from subsequent lethal challenge, suggesting an important but as yet unknown *in vivo* role for these structures. To understand the gene products involved in OMV and tube production, we performed a genetic screen using the two-allele transposon mutant library of *F. novicida*, identifying a number of hyper- and hypo-vesiculating mutants. As has been reported for other Gram-negative bacteria, mutations that affect envelope integrity result in hyper-vesiculation. Hypo-vesiculating mutants were isolated in genes that function in central aspects of carbon metabolism, as well as in genes of unknown function. Using several experimental approaches, we identified links between the hypo-vesiculating mutants and the response of *Francisella* to oxidative stress. Our results suggest an important correlation between oxidative stress, carbon metabolism, and the regulated production of OMV and tubes by *F. novicida*. We propose that *Francisella* senses the host cell oxidative burst as a signal to turn on a virulence program, leading to the production of OMV and tubes. Furthermore, as we have found that OMV and tube production is conserved in the *F. tularensis* SchuS4 strain, our results can provide a framework to understand virulence mechanisms of human pathogenic *Francisella*.

Session 3:

Short presentations

Session 3: Short Presentations

Chairs: Monique Barel
INSERM U1151, France

David Thanassi,
Stony Brook University, USA

S3-1 On how tularemia entered and found its way through Western Europe – a genomic epidemiology study
Chinmay Dwibedi, Adrian Lärkeryd, Kerstin Myrtennäs, Caroline Öhrman, Dawn Birdsell, Elin Nilsson, Edvin Karlsson, Christian Hochhalter, Andrew Rivera, Sara Maltinsky, Brittany Bayer, David Wagner, Paul Keim, Holger C Scholz, Herbert Tomaso Matthias Wittwer, Paola Pilo, Marta Hernández Pérez, David Rodriguez-Lazaro, Mats Forsman, Pär Larsson, Anders Johansson

S3-2 Towards a global phylogeography of *Francisella tularensis* subspecies *holarctica*
K. Myrtennäs, D. Birdsell, C. Öhrman, A. Lärkeryd, C. Dwibedi, D. Elfsmark, P. Lindgren, P. Larsson, A. Sjödin, P. Stenberg, E. Nilsson, E. Karlsson, M. Byström, S. Bäckman, M. Granberg, A. Macellaro, C. Hochhalter, A. Rivera, C. Mitchell, J. Sahl, M. Niemcewicz, K. Marinov, H. Tomaso, R. Grunow, K. Heuner, H. Scholz, S. Nikkari, S. Sissonen, M. Gyuranecz, P. Keim, D. Wagner, A. Johansson, M. Forsman

S3-3 DNA supercoiling activity of DNA gyrases from *Francisella tularensis* strains resistant to quinolones
Patricia Renesto and Max Maurin

S3-4 Proteomic analysis of *Francisella tularensis* subspecies *holarctica* isolates from patients and environment in tularemia epidemics
Aynur Karadenizli, Murat Kasap, Doğanhan Kadir Er, Abula Ayimugu, Kübra Karaosmanoglu, Gürler Akpınar, Hüseyin Uzuner

S3-5 Loss of O-antigen and deglycosylation of the capsule-like complex promote biofilm formation in *Francisella tularensis*
Anna Champion, Kelly Freudenberger, Nrusingh Mohapatra, Aloka Bandara, and Thomas J. Inzana

S3-1

On how tularemia entered and found its way through western Europe – a genomic epidemiology study

Chinmay Dwibedi[1, 2], Adrian Lärkeryd[2], Kerstin Myrtennäs[2], Caroline Öhrman[2], Dawn Birdsell[3], Elin Nilsson[2], Edvin Karlsson[2], Christian Hochhalter[3], Andrew Rivera[3], Sara Maltinsky[3], Brittany Bayer[3], David Wagner[3], Paul Keim[3], Holger C Scholz[4], Herbert Tomaso[5], Matthias Wittwer[6], Paola Pilo[7], Marta Hernández Pérez[8], David Rodriguez-Lazaro[8], Mats Forsman[2], Pär Larsson[2], Anders Johansson[1]

[1] Umeå University, Umeå, Sweden; [2] Swedish Defence Research Agency, Umeå, Sweden; [3] Northern Arizona University, Flagstaff, Arizona, USA; [4] Bundeswehr Institute of Microbiology, Munich, Germany; [5] Friedrich-Loeffler-Institut Federal Research Institute for Animal Health, Jena, Germany; [6] Spiez Laboratory, Federal office for Civil Protection, Spiez, Switzerland; [7] University of Bern, Bern, Switzerland; [8] Universidad de Burgos, Burgos, Spain; Department of clinical microbiology, Clinical bacteriology, and Laboratory for molecular infection medicine Sweden (MIMS), Umeå University, SE-901 85 Umeå, Sweden, chinmay.dwibedi@umu.se

Objective. Over the last few decades, tularemia has been increasingly reported in continental Western Europe, with Spain recording its first case only in 1996. Recent work indicates that the disease is caused by a specific subpopulation of *Francisella tularensis holarctica*. Little is known about this seemingly successful subpopulation. Here, we sought to understand the origin of this subpopulation, phylogenetic diversity, evolutionary history, and how it spreads across Europe.
Methods. Sixty-seven whole genome sequences of *F. tularensis* from continental Western Europe, isolated between 1947 and 2013, were analyzed and compared with sequences of global origin. Using canonical single nucleotide polymorphisms identified from the genomic analyses an additional 155 isolates were genotyped. To understand the natural mutation rate we carried out a Bayesian analysis on isolates from Spain.
Results. All isolates from continental Western Europe were members of specific genetic clade of *F. tularensis.* Two genetic branches were identified: the first clade exhibited a star phylogeny and was observed throughout the region and the second clade was geographically restricted to the Eastern boundary of the study region. Region specific localization of sub-clades was observed. Higher genetic diversity was observed in the Eastern boundary of the study region as compared to the Western boundary. Comparison with historical outbreaks revealed that tularemia endemic regions have persisted over 60 years and expanded locally over time. The data suggested that *F. tularensis* has moved large geographical distances with little genetic changes. A mutation rate of 0.4 mutations per year was identified.
Conclusion. The results of this study are consistent with recent clonal spread of *F. tularensis* throughout continental Western Europe, starting from East and moving

to the West. The restricted genetic diversity over a 67 year time span and the low natural mutation rate suggest a life-cycle of the bacterium with long periods of dormancy or an extremely low replication rate. The work also provides a model for how *F. tularensis* is occupying new land areas. The model contains two distinct components: first it seems that when the bacterium successfully establishes in an area it remains there while slowly accumulating genetic diversity and locally expanding its geographical range; second there is a large distance movement from these focal points explaining how nearly identical genomes may be found in distant places.

S3-2

Towards a global phylogeography of *Francisella tularensis* subspecies *holarctica*

K. Myrtennäs[1*], D. Birdsell[2*], C. Öhrman[1], A. Lärkeryd[1], C. Dwibedi[1,3], D. Elfsmark[1], P. Lindgren[1], P. Larsson[3], A. Sjödin[1], P. Stenberg[1], E. Nilsson[1], E. Karlsson[1], M. Byström[1], S. Bäckman[1], M. Granberg[1], A. Macellaro[1], C. Hochhalter[2], A. Rivera[2], C. Mitchell[2], J. Sahl[2], M. Niemcewicz[4], K. Marinov[5], H. Tomaso[6], R. Grunow[7], K. Heuner[7], H. Scholz[8], S. Nikkari[9], S. Sissonen[10], M. Gyuranecz[11], P. Keim[2], D. Wagner[2], A. Johansson[3], M. Forsman[1]

[*]These authors contributed equally to this work.

[1]Swedish Defence Research Agency, Umeå, Sweden; [2]Northern Arizona University, Flagstaff, USA; [3]Umeå University, Umeå, Sweden; [4]Military Institute of Hygiene & Epidemiology, Poland; [5]Military Medical Academy, Sofia, Bulgaria; [6]Friedrich-Loeffler-Institut Federal Research Institute for Animal Health, Jena, Germany; [7]Robert Koch Institute, Berlin, Germany; [8]Bundeswehr Institute of Microbiology, Munich, Germany; [9]Centre for Military Medicine, Helsinki, Finland; [10]National Institute for Health and Welfare, Helsinki, Finland; [11]Institute for Veterinary Medical Research, Budapest, Hungary; Kerstin Myrtennäs, Swedish Defence Research Agency, Division of CBRN Defence and Security, SE-901 82 Umeå, Sweden. E-mail: kerstin.myrtennas@foi.se.

Previous phylogeographic studies of *Francisella tularensis* subspecies *holarctica*, the tularemia type B bacterium, included strain sets with limited diversity and geographical distribution, and short time spans. Therefore, to better assess global evolutionary trends, we analyzed whole genome sequence variation within a dataset of 888 *F. tularensis* type B samples collected from three continents over 80 years. Together, the 561 new sequenced isolates, 76 previously published genomes, and 251 laboratory typed samples, represent 28 countries and 43 host species. There was an overrepresentation of samples from humans (n=398), the time period 2000-2013 (n=490), and samples from Scandinavia (n=355). The phylogenetic analyses revealed additional diversity within type B but confirmed that Francisella tularensis subspecies holarctica is a clonal pathogen with limited overall variation. We herein extended the global phylogenetic framework for defining distinct genetic clades with 88 new canonical single nucleotide polymorphisms (canSNPs) to include a total of 131 canSNPs within *Francisella tularensis* subspecies *holarctica.* A combined phylogenetic analysis on SNPs and other genomic differences, such as insertions/deletions and variable repetitive regions, provided maximal information on the phylogenetic variation. The spatial analysis revealed that the global expansion of a large group of strains, denoted B.3 clades, is extensive and closely related isolates are commonly widely distributed in North America and Eurasia. Similar expansion pattern have been observed for the most basal clade B.16 that consists of strains previously described as biovar japonica. This group was originally thought to be

confined to the Japanese islands, but has recently been identified in Australia, China and Turkey. Further, the genomic analyses revealed that two members of another clade with basal position, the B.2/3 clade, currently isolated only from California, are quite distant (>200 SNPs) to members of its B.3 sister clade. Functional analysis on each clade may reveal clues regarding different ecological niches and signs of convergent or parallel evolution (ongoing). In conclusion, this study provides comprehensive information on the global phylogenetic diversity in *Francisella tularensis* subspecies *holarctica* connected to time, geography and source. We provide a unified genetic nomenclature for canSNPs to allow efficient communication among researchers.

S3-3

DNA supercoiling activity of DNA gyrases from *Francisella tularensis* strains resistant to quinolones

Patricia Renesto[1] and Max Maurin[1,2]

[1] LAPM, CNRS/UJF UMR 5163, Université Grenoble Alpes, Grenoble, France, [2] Centre National de Référence des Francisella, Institut de Biologie et de Pathologie, Département des Agents Infectieux, Centre Hospitalier Universitaire de Grenoble, France

Quinolones are one of the most commonly prescribed classes of antibacterials agents in the world and are used to treat several bacterial infections in humans. Accordingly, microbiological and clinical data showed that ciprofloxacin, and possibly other fluoroquinolones represent an efficient first-line treatment for oral therapy of tularemia, a disease caused by the Gram negative bacterium *Francisella tularensis*. These compounds inhibit DNA synthesis through interaction with complexes composed of DNA and either of the two target enzymes, DNA gyrase and topoisomerase IV that belong to type IIA topoisomerases. A collection of fluoroquinolones resistant clones of *Francisella* was generated in our lab through an experimental evolution protocol applied on sensitive strains exposed to increasing fluoroquinolone concentrations. Exposure to antibiotic was accompanied by mutations in GyrA and GyrB. While some mutations were restricted to discrete regions of the so-called quinolone-resistance-determining regions (QRDR), amino acid substitutions or deletions never previously reported were also identified. Here, our aim was to further investigate the role of such newly identified GyrA and GyrB mutations in fluoroquinolone resistance. Recombinant WT mutated GyrA and GyrB subunits from *Francisella novicida* and *Francisella philomiragia* were expressed in *E. coli* and purified soluble proteins were used to reconstitute highly active gyrase complexes. First, and as assessed by supercoiling activities using an *in vitro* assay, we determined if mutations alter the enzymatic function of the DNA gyrase. In a second step, and in order to prove the role of mutations in fluroquinolone resistance, MICs and DNA gyrase inhibition were determined for ciprofloxacin and moxifloxacin. This approach demonstrated that, as previously shown for other human pathogens, DNA gyrase mutations are not necessarily related to fluoroquinolones resistance.

S3-4

Proteomic analysis of *Francisella tularensis* subspecies *holarctica* isolates from patients and environment in tularemia epidemics

Aynur Karadenizli[1], Murat Kasap[2], Doğanhan Kadir Er[1], Abula Ayimugu[3],Kübra Karaosmanoglu[3], Gürler Akpınar[2], Hüseyin Uzuner[1]

[1] Kocaeli University Medical Faculty, Medical Microbiology Department, Kocaeli, Turkey; [2] Kocaeli University Medical Faculty, Medical Biology Department, Kocaeli, Turkey; [3] Kocaeli University Technology Faculty, Department of Biomedical Engineering, Kocaeli, Turkey

Francisella tularensis is the causative agent of tularemia. Recently, the cases has been increasingly observed for the last decade in Turkey. However, only limited studies related to molecular characteristics of these naturally occurring strains in Turkey were performed. Therefore, in this study, we investigated the protein profile of the *F. tularensis* subsp. *holarctica* strains isolated from clinical and the environmental samples using two-dimensional gel electrophoresis (2DE). For comparison, three strains isolated from clinical samples and an environmental strain isolated from trough water from different locations in Turkey was used in addition to *F.tularensis* subsp. *holarctica* NCTC 10857. The bacteria were grown in cation-adjusted Mueller Hinton broth containing isoVitalex, ferric pyrophosphate and glucose. Cells were collected at mid-log phase and protein extracts were prepared in 2DE lysis buffer using a bead beater. After 2DE and gel staining, 175±7 well-resolved protein spots were analyzed. By using normalized protein spot intensities, PCA analysis was performed. The results indicated that the strain isolated from water source was different then the strains isolated from the patients. Most interestingly, all Turkish strains were strikingly distinguishable from the standard NCTC strain. Regulations were detected in 16 spots when two-fold regulation criteria were applied. These spots were subjected to MALDI-TOF/TOF analysis and identified. Classification of the identified proteins based on the metabolic function revealed that the translation machinery was the most varying metabolic process among the isolates.

S3-5

Loss of O-antigen and deglycosylation of the capsule - like complex promote biofilm formation in *Francisella tularensis*

Anna Champion, Kelly Freudenberger, Nrusingh Mohapatra, Aloka Bandara and Thomas J. Inzana

Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Life Sciences 1, 970 Washington St., Blacksburg, VA USA 24061

Francisella tularensis is capable of living in diverse environments that include ticks, water, mud, within amoebae, and within macrophages and other cells of most mammals. Bacteria that survive and persist in diverse environments normally make a biofilm as a protective mechanism against unsuitable growth conditions. *F. novicida*, considered a subspecies of *F. tularensis,* makes an excellent biofilm when grown in broth under static or flow cell conditions within 5 days incubation. In contrast, *F. tularensis* makes a poor biofilm, if any, under the same growth conditions and time period. However, the live vaccine strain (LVS) makes a substantial biofilm after 15 days incubation. Furthermore, mutants of LVS that lack lipopolysaccharide O-antigen (LVSΔ*wbtI*) and/or cannot glycosylate proteins that form the capsule-like complex (CLC) (LVSΔ1423-1422) made 2 to 4-fold more biofilm than LVS. Attachment to polystyrene wells by a double mutant lacking O-antigen and glycosylation of CLC (LVSΔ*wbtI*Δ1423-22) was significantly better (p=0.01) than the parent or either single mutant alone after 1 hr incubation. LVSΔ*wbtI*Δ1423-22 also produced ~15-fold more biofilm that was thicker and more confluent than the biofilm from LVS, as determined by confocal laser scanning microscopy. Growth in Chamberlains defined medium (CDM) promoted biofilm formation more than growth in modified Mueller-Hinton medium (MMH), but the difference was most pronounced by double mutant LVSΔ*wbtI*Δ1423-22. In contrast, biofilm formation was not significantly enhanced by *F. novicida* O-antigen and CLC glycosylation mutants compared to the parent, but was more prominent when the parent or mutants were grown in CDM compared to MMH. Biofilms normally consist of a polysaccharide matrix. Therefore, enhanced biofilm formation by O-antigen and CLC-glycosylation mutants was unexpected. Biofilms were collected, extracted for exopolysaccharide (EPS), and analyzed by gas chromatography/mass spectrometry of their per-O-trimethylsilyl derivatives, and by glycosyl linkage analysis. The EPS was determined to be greater than 99% 1-4 linked glucose. The biofilm was also strongly reactive with fluorescein-tagged Concanavalin A (reactive with α-D glucose or mannose), but not with fluorescein-tagged wheat germ agglutinin (reactive with *N*-acetylglucosamine). Similar results were obtained with Type A *F. tularensis*, including enhanced biofilm formation by a Type A O-antigen mutant. In conclusion we have shown that the absence of O-antigen and protein glycosylation significantly enhances biofilm formation in *F. tularensis*, but not in *F. novicida*, and that a glucose polymer forms the biofilm matrix. Carbohydrate components on the bacterial surface may sterically interfere with adherence by adhesins, or inhibit EPS formation in *F. tularensis*.

Session 4:

Poster Session A

Session 4: Poster Session A

S4-1 The changes in *F. tularensis* proteome in response to the deletion of dsbA-like gene determined by SILAC approach
Pavkova Ivona, Klimentova Jana, Schmidt Monika, Sheshko Valeria and Stulik Jiri

S4-2 *Francisella tularensis* subsp. *holarctica* (strain FSC200) outer membrane nanotubes – proteomic characterization
Jana Klimentova and Jiri Stulik

S4-3 Coordinate control of virulence gene expression in *F. tularensis* involves direct interaction between key regulators
Amy E. Rohlfing and Simon L. Dove

S4-4 Diagnostic shotgun metagenomics of cervical lymph node specimens of tularemia patients identified *Francisella tularensis* and previously unnoticed co infections
Birdsell D, Özsürekci Y, Rawat A, Aycan A.E., Gurbuz V, Mitchell C, Sahl J, Johansson A, Colman R.E., Schupp J, Ceyhan M, Keim PS and Wagner DM

S4-5 A wgMLST approach to *Francisella tularensis* subspecies identification
Kitty Chase, April Shea, William Dorman and Mark Wolcott

S4-6 Serological proteome analysis of *F. tularensis*
Hila Cohen, Erez Bar-Haim, Shahar Rotem, Uri Elia, Ofer Cohen and Theodor Chitlaru

S4-7 Proteomic studies of atypical *Francisella tularensis* strains isolated from natural foci in Georgia
M.Meparishvili, E.Mikautadze, M. Nozadze, R. Solomonia, G.Babuadze, E. Zhgenti, G. Chanturia, M. kekeklidze , R. Obiso and S. Francesconi

S4-8 Canfindit – an *in silico* clade identification tool for *Francisella tularensis*
Eric Zuchantke, Röbbe Wünschiers, Mats Forsman and Herbert Tomaso

S4-9 Analysis of *Francisella tularensis* protein O-glycosylation pathway
Lucie Balonova, Marek Link, Vera Dankova and Jiri Stulik

S4-10 The survey of wild animals for tularemia in Poland
W. Iwaniak, K. Szulowski, M. Szymajda, J. Złotnicka and M. Kubajka

S4-11 *Francisella novicida* responds to *Burkholderia* diffusible signal factor (BDSF) interspecies signaling to disperse biofilm and increase siderophore production
Scott N. Dean, Myung-Chul Chung and Monique L. van Hoek

S4-12 The role of OxyR in oxidative stress response of *Francisella tularensis* LVS
Marie Honn, Helena Lindgren and Anders Sjöstedt

S4-13 Defining the host-derived carbon sources and metabolic pathways required for *Francisella tularensis* intracellular and *in vivo* growth
Lauren Radlinski, Jason Brunton, Shaun Steele, Sharon Taft-Benz and Tom Kawula

S4-14 see S1-3

S4-15 see S1-4

S4-16 see S1-5

S4-17 see S2-4

S4-18 see S3-1

S4-19 see S3-2

S4-20 see S3-3

S4-21 see S3-4

S4-22 see S3-5

S4-1

The changes in *F. tularensis* proteome in response to the deletion of dsbA - like gene determined by SILAC approach

Pavkova Ivona, Klimentova Jana, Schmidt Monika, Sheshko Valeria and Stulik Jiri

Department of molecular pathology and biology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575. Hradec Kralove, Czech Republic

The conserved hypothetical lipoprotein (FTT1103, FTL_1096, FTS_1067) with homology to bacterial DsbA proteins has previously been demonstrated to be required for intracellular replication and animal death. Furthermore the *dsbA* deletion mutant was able to protect mice against the virulent *F. tularensis* subsp. *holarctica* strain FSC200. In *Francisella*, this protein seems to be quite unique as it is a glycosylated lipoprotein and in addition to its disulfide oxidoreductase activity it seems to modulate isomerase and chaperone activity. It can be expected from the multifunctional role that the *F. tularensis* DsbA affects the function of a wide range of membrane and secreted proteins including essential virulence factors. And thus its observed role in virulence might rather be as a consequence of disruption of other “true” virulence determinants. In our previous studies we applied both gel-based and non-gel based proteomic approaches in order to monitor the global changes in fractions enriched for membrane proteins of *F. tularensis* LVS with *dsbA* gene deletion. Using the 2-DE + MS and iTRAQ+LC-MS/MS we were able to identify only 9 proteins with significant distinct abundance compared to the wild type strain. Here we present new data obtained from the SILAC-based quantitative proteomic approach. This analysis enables the identification of 63 proteins with significantly altered amounts in the *dsbA* mutant strain compared to the wild type strain. Of them, 28 proteins showed higher levels and 35 proteins lower levels in the mutant strain. These proteins represent quite heterogeneous group including hypothetical proteins, proteins associated with membrane structures and potential secreted proteins. Many of them have already been known to be associated with *F. tularensis* virulence. Several proteins were selected for further studies focused on their potential role in pathogenesis of tularemia.

S4-2

Francisella tularensis subsp. *holarctica* (strain FSC200) outer membrane nanotubes – proteomic characterization

Jana Klimentova and Jiri Stulik

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence,Trebesska 1575, Hradec Kralove 500 01, Czech Republic

Outer membrane vesicles (OMV) secreted by gram-negative bacteria play an important role in bacterial physiology, as well as in virulence and host-pathogen interaction. Their immunomodulatory potential in some bacteria has also been reported. It has been described previously that *F. novicida* apart from the classical spherical OMV produces also less usually shaped outer membrane tubes (McCaig et al. 2013). In this study OMV were successfully isolated from culture medium of *F. tularensis* subsp. *holarctica* FSC200 by medium concentration followed by high-speed centrifugation. Transmission electron microscopy revealed a rich mixture of spherical and tubular vesicles on the background of other extracellular material such as high molecular weight protein complexes and aggregates or pili. This mixture was further separated by density gradient centrifugation and two fractions were collected. Lower density fraction contained pure OMV (spherical and tubular all together), higher density fraction contained the non-vesicular material where chaperones were the most common component. In the purified OMV fraction the tubes tended to crumble into spheres as a result of long-term storage or freezing and thawing of the sample. It was thus not clear whether the bacterium produced two distinct types of vesicles respectively, or the spheres originated from the tubes by sample handling. Proteomic analysis of the pure OMV fraction revealed ca 300 proteins. This fraction was rich in outer membrane proteins, lipoproteins, immunoreactive proteins as well as previously described virulence factors. The most prominent protein in higher density fraction was GroEL but other virulence factors like IglC tended to peak there as well, suggesting that they were probably not contained in the vesicles and were secreted by a different mechanism.

S4-3

Coordinate control of virulence gene expression in *F. tularensis* involves direct interaction between key regulators

Amy E. Rohlfing and Simon L. Dove

Division of Infectious Diseases, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts, 02115, USA

The expression of genes necessary for the intramacrophage growth and virulence of *F. tularensis* is positively regulated by a putative DNA-binding protein, PigR, and the SspA family members, MglA and SspA. MglA and SspA form a heteromeric complex that associates with RNA polymerase (RNAP); it is thought the ability of the MglA-SspA complex to associate with RNAP is critical for the regulatory function of MglA and SspA. Evidence suggests that PigR is able to directly interact with the MglA-SspA complex, although it was unknown whether the interaction between PigR and the MglA-SspA complex is necessary for PigR to regulate gene expression. Here, using a combination of genetic assays, we identified mutants of MglA and SspA that are specifically defective for interaction with PigR. Analysis of these MglA and SspA mutants in *F. tularensis* found that the mutants are unable to support expression of MglA, SspA, and PigR regulated genes. Our work indicates that a direct interaction between the MglA-SspA complex and PigR is critical for the ability of PigR to function as a transcription activator. Our findings have also identified a surface of the MglA-SspA complex that is important for the interaction with PigR and support the idea that PigR exerts its regulatory effects through a direct interaction with the RNAP-associated MglA-SspA complex.

S4-4

Diagnostic shotgun metagenomics of cervical lymph node specimens of tularemia patients identified *Francisella tularensis* and previously unnoticed co-infections

Birdsell D.[1], Özsürekci Y.[2], Rawat A.[3], Aycan A.E.[2], Gurbuz V.[2], Mitchell C.[1], Sahl J.[1,4], Johansson A.[5], Colman R.E.[4], Schupp J.[4], Ceyhan M.[2], Keim P.S.[1,4], Wagner D.M.[1]

[1] Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA; [2] Hacettepe University Faculty of Medicine, Department of Pediatric Infectious Disease Unit in Ankara, Turkey, [3] Translational Genomics Research Institute, Phoenix AZ, USA, [4] Translational Genomics Research Institute, Flagstaff AZ, USA, [5] Department of Clinical Microbiology, Clinical Bacteriology, and the Laboratory for Molecular Infection Medicine Sweden, Umeå University, Umeå, Sweden

Tularemia, caused by the bacterium *Francisella tularensis*, continues to be on the rise in Turkey. The majority of patients get infected by contaminated drinking water and presents with infection of the throat with swollen lymph nodes of the neck and under the jaw. In this study, we investigated 14 patients diagnosed early after onset of disease. Despite prompt antibiotic treatment, these patients suffered from protracted disease symptoms. To identify possible factors to explain for the poor treatment response to antibiotics we conducted an unbiased molecular survey by direct whole genome sequencing of DNA extracts from neck lymph nodes from 8/14 patients. The resulting shotgun metagenomic data were searched for microbe sequences (bacterial and viral) and bacterial genes or mutations known to confer antibiotic resistance. *F. tularensis* sequences were detected in all samples along with extremely low levels of the non-pathogenic bacteria *Ralstonia picketti* and *Propionibacterium acnes*. In addition, both Hepatitis B virus (HBV) and Human Parvovirus B-19 were detected in one single individual and only Human Parvovirus B-19 was detected in two other individuals. Subsequent diagnostic PCR confirmation verified the metagenomic findings of HBV and Human Parvo B-19 virus, The HBV status in one patient was independently confirmed through serological diagnostics, despite evading notice during initial patient medical examination. This study illustrates the capability of diagnostic shotgun metagenomics to effectively detect *F. tularensis* and other pathogenic microbes in lymph node samples. We found insufficient evidence for the involvement of antibiotic resistance genes to explain the poor response to antibiotic treatment. Similarly, the protracted symptoms in these tularemia patients could not be conclusively linked with the presence of co-infections. The analysis however brought to light that diagnostic shotgun metagenomics readily detects multiple pathogens including *F. tularensis* in the acute phase of disease. The application of diagnostic shotgun metagenomics promises to advance our understanding of complex infectious disease presentations.

S4-5

A wgMLST approach to *Francisella tularensis* subspecies identification

Kitty Chase, April Shea, William Dorman, Mark Wolcott

U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD U.S.A.

Francisella tularensis has been divided into four distinct subspecies based on biochemical, phenotypic, and virulence traits: subsp. *tularensis* (type A), subsp. *holarctica* (type B), subsp. *novicida* and subsp. *mediasiatica*. The identification and differentiation of these has been challenging as *F. tularensis*, as a species, has greater than 99.2% sequence similarity. Traditional biochemical methods of subspecies identification, or sequence typing, are labor intensive and serve as a potential source of contamination as well as are a significant risk of laboratory infection. Molecular methods for sequence typing can provide critical insights for outbreak analysis and population variation. Genomic methods, such as restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis, and ribosomal multi-locus sequence typing (rMLST) (16S and 23S) give further info to genetic rearrangements, but these methods rely on conserved regions so emerging pathogens, or differentiation of close genetic neighbors, could be missed. Next-generation sequencing allows for use of the entire genome for the identification of loci to be used for sequence typing in a more cost effective manner. Whole genome multi-locus sequence typing (wgMLST) looks outside of traditional housekeeping genes to span the whole genome for allelic variation (SNPs, indels, recombinations). By using 2000-4000 loci as compared to 7 housekeeping loci with traditional MLST schemes, a greater sequence typing resolution can be achieved. A F. tularensis wgMLST pipeline is being developed using commercial software (BioNumerics 7.5 software; Applied Maths NV). Alleles are identified using Fastq files with two approaches: de novo assembly followed by a BLAST search and assembly-free identification directly from the sequence reads. All calculations are done in the cloud so very limited local computational usage or memory is needed. Alleles for different subspecies are generated and compared with current alleles in known sequence types. Identification and characterization is done by the assigning sequence types to these allelic profiles. In addition, new alleles can be submitted for curation into the current scheme. This approach allows multiple schemes to be generated, such as core genome-based, ribosomal, as well as multiple subsets based on specific genome data, such as virulence factors. Next-generation sequencing and wgMLST will provide another identification and characterization method that will extend our capabilities to understand this organism better.

S4-6

Serological proteome analysis of *F. tularensis*

Hila Cohen, Erez Bar-Haim, Shahar Rotem, Uri Elia, Ofer Cohen, Theodor Chitlaru

Biochemistry and Molecular genetics Department, The Israel Institute for Biological Research, Israel

With the objective of identifying immuno-dominant bacterial antigens expressed upon exposure of experimental animals to *F. tularensis*, we conducted a serological-proteome analysis (SERPA) which included: (a) 2-DE separation of protein-extracts prepared from in vitro-cultured stationary *F. tularensis* cells, (b) MS-MS assisted identification of abundant proteins and (c) 2-DE Western-blot probing of proteins with a repertoire of sera collected from mice or rabbits immunized with *F. tularensis* of the LVS or Schu4 strains. These experimental animals were immunized by a variety of live, attenuated or inactivated bacterial cultures, by different routes of administration, enabling identification of about 30 bacterial antigens; several of these were not previously documented as immunogens. The kinetics of the sero-convertion process of the infected animals was determined with respect to several major antigens. The potential significance of the immunogens identified in this study in the pathogenesis of *F. tularensis* as well as their possible relevance for the development novel diagnostic, disease progression biomarkers and therapeutic strategies is discussed.

S4-7

Proteomic studies of atypical *Francisella tularensis* strains isolated from natural foci in Georgia

M.Meparishvili[1], E.Mikautadze[1], M. Nozadze[1], R. Solomonia[1], G.Babuadze[2], E. Zhgenti[2], G. Chanturia[2], M. kekeklidze[2] , R. Obiso[3], S. Francesconi[4]

[1] Institute of Chemical Biology, Ilia State University; [2] National Center for Disease Control (NCDC), Tbilisi, Georgia; [3] Attimo Research and Development, USA, [4] Naval Medical Research Center, Silver Spring, MD, USA

Tularemia is a zoonotic infection caused by *Francisella tularensis* - a small, faintly-staining, Gram-negative, non-motile, non-spore-forming, pleomorphic bacterium, typically appearing as short rods or coccoid forms. Two regions in the Republic of Georgia with naturally occurring tularemia foci have been identified: one in the mountainous Meskhet-Javakheti region and another in the Kartl-Kakheti Valley. Currently the pathogen repository at the National Center for Disease Control and Prevention contains more than 100 *F. tularensis* isolates collected in Georgia throughout the past 60 years. Genomic diversity is common amongst isolates of *F. tularensis* and genetic studies with our group have indicated some unique features of Georgian isolates. To further expand this knowledge, we have applied a proteomic based analysis of six strains of *F. tularensis* from Georgia (strains: 4809; 4811; 4812; 967; 8932 and 6958, using the LVS strain as a reference strain). Bacterial cells were collected at the logarithmic phase of growth and samples were prepared for 2-dimensional gel-electrophoresis. Images of silver stained gels were analyzed by special software and consistently differentially bands identified. Mass-Spectrometry analysis of these differentially expressed proteins is on-going but shows that certain strains of *F. tularensis* require further investigation and show unique proteomic features.

S4-8

Canfindit – an *in silico* clade identification tool for *Francisella tularensis*

Eric Zuchantke[1], Röbbe Wünschiers[1], Mats Forsman[2], Herbert Tomaso[3]

[1] Biotechnology / Computational Biology, University of Applied Sciences, Mittweida, Germany; [2] Swedish Defence Research Agency, Umeå, Sweden; [3] Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Jena, Germany

Canonical single nucleotide polymorphisms (canSNPs) and InDels are used to analyze the spread of *Francisella tularensis* and for identification of single clades. By using methods like Multiple Loci VNTR Analysis (MLVA) these single nucleotide polymorphisms can be identified. For the identification of each canSNP at least two primer pairs or markers are needed to distinguish between the ancestral and derived state. Because this wet-lab procedure is time consuming and expensive when applied to a wide range of canSNPs, the use of computational approaches is an emergent step. Beside the analysis of single sequence parts with MLVA, next generation sequencing (NGS) is also performed. By using the generated information from *F. tularensis* sequencing, we created a time and cost efficient tool for the identification and assignment of single clades, called CanFindIt. For testing eleven genome samples of *Francisella tularensis* subsp. *holarctica* were analyzed. These samples were previously genotyped and analyzed by using nine canSNPs and two InDels. CanFindIt found all eleven experimentally detected markers correctly. It uses raw Fastq-files of a single sequencing result and available marker sequences, only. By knowing the assignment of each sample to the clades, an automatic comparison to given reference sequences is performed to create a phylogenetic tree. The created tool CanFindIt is written in Python and uses advantages of several other tools. These tools are MEGA for calculating the phylogenetic tree, progressiveMauve for the sequence alignment and CanSNPer as reference for the canonical SNPs. CanFindIt can be used for a quick, user guided clade identification and classification for known canSNPs and InDels of *Francisella tularensis*.

S4-9

Analysis of *Francisella tularensis* protein O-glycosylation pathway

Lucie Balonova, Marek Link, Vera Dankova, Jiri Stulik

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, Faculty of Military Health Sciences, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

Glycosylation is one of the most frequent post-translational modifications of proteins occuring in all domains of life – eukarya, archaea, and bacteria. Glycans are known to influence the protein properties and may also participate in the processes of cell-to-cell recognition, host cell adhesion, or even modulate immune responses. Their potential role as mediators of host-microbe interactions has heightened an interest in investigating glycosylation in bacterial pathogens. Interestingly, several pathogenic bacteria possess functional glycosylation apparatus, by which they modify their proteins. A functional glycosylation apparatus has recently been revealed also in *Francisella tularensis*. Our current knowledge of *Francisella* glycoproteome is limited to the three glycoproteins, all of them being required for *F. tularensis* virulence. Unveiling the biological significance of glycosylation requires both characterization of subset of protein substrates targeted for glycosylation and defining the glycoprotein biosynthetic machinery, following the consequences of its disruption. Enzymes involved in the Gram-negative bacterial glycosylation pathway can generally be divided into several classes according to their function. First class comprises the proteins that are required for the biosynthesis of particular monosaccharides, from which the final glycan is to be composed, e.g. dehydratases, aminotrasferases, epimerases, and acetyltransferases. Second class is represented by the glycosyltransferases, which mediate a transfer of the synthesized monosaccharide units from the nucleotide-activated sugar donors to the growing oligosaccharide chain. The final oligosaccharide is then flipped from the cytosol across the inner membrane to the periplasm by the action of the so-called flippase. Glycoprotein biosynthesis is finalized by the *en bloc* transfer of the newly synthesized glycan to the nascent protein in the periplasm. The glycan transfer is mediated by an oligosaccharyltransferase. *F. tularensis* oligosaccharyltransferase PglA is the only hitherto experimentally proven enzyme of the microbe´s glycomachinery. The study presented here is aimed to define protein glycosylation pathway and to evaluate its biological significance in *Francisella* pathogenesis. For this purpose, mutant strains with disrupted genes encoding proposed glycosylation-related proteins are constructed by targetron insertional mutagenesis. The changes in glycan structure are determined by means of mass spectrometry and the effect of genes disruption on *Francisella* pathogenesis is monitored by various biological assays. By combining gene disruption and mass spectrometry approaches, involvement of each selected gene product in the glycosylation machinery may be demonstrated and the role of the proteins, encoded by disrupted genes, in the glycosylation pathway of *F. tularensis* is proposed.

S4-10

The survey of wild animals for tularemia in Poland

W. Iwaniak, K. Szulowski, M. Szymajda, J. Złotnicka, M. Kubajka

National Veterinary Research Institute, Department of Microbiology, Al. Partyzantow 57, 24-100 Pulawy, Poland

Tularemia in Poland has not been truly investigated up to 2014. Almost every year there are several tularemia cases in humans but there was lack of an unequivocal and strong evidence for the presence of the etiological agent, i.e. *Francisella* (F.) *tularensis* in wild animal reservoir. We have started the monitoring of tularemia in wild animals in the frame of the National Programme financed by Polish Ministry of Agriculture and Rural Development. The aim of the studies carried on in 2014 was evaluation of occurrence of *F. tularensis* infections in free-living animals by use a Real-Time PCR as well as classical culture. Samples (479) coming from wild animals (wild boars, red deers, European brown hares, mole, Eurasian Red Squirrel) were tested. The samples were collected during 2014 from hunted and found dead animals. We collected internal organs like spleen, liver and lungs and stored them at -80°C before testing. After defrosting, the organ samples were homogenized as soon as possible and about 50 µl of the homogenates of each organ sample was transferred on Cystine Heart Agar (Becton Dickinson). The same samples were inoculated also on selective medium - Cystine Heart Agar with VCN Selective Supplement (OXOID) addition. Suspected colonies were identified as *F. tularensis* by serological, microscopical, biochemical and molecular methods. The template DNA was prepared in accordance with the manufacturer's protocol (Qiagen). The QuantiTect Probe PCR Kit was used to perform the Real-Time PCR targeting the gene encoding 23-kDa protein derived from *F. tularensis.* Analytical specificity was confirmed, testing 40 bacterial strains other than *F. tularensis.* Moreover, the diagnostic specificity was 100%, when evaluated on 100 organ samples of apparently healthy and hunted wild boars. Diagnostic sensitivity was assessed on *F. tularensis* control strains (ATCC 6223, LVS) as well as *F. tularensis* strains derived from the NVRI collection. Additionally, the tissue samples contaminated with *F. tularensis* control strains, were used to confirm the diagnostic sensitivity. The samples (lungs, spleen, liver) taken from one dead hare, were found positive both in Real-Time PCR and in culture. The isolate was identified as a *F. tularensis ssp. holarctica.* The remaining samples gave negative results both in Real-Time PCR and in culture. The isolation of the *F. tularensis ssp. holarctica* is an undeniable evidence for the presence of the agent in the environment. It is also the first documented report of isolation and identification of the *F. tularensis ssp. holarctica* in Poland.

S4-11

Francisella novicida responds to *Burkholderia* diffusible signal factor (BDSF) interspecies signaling to disperse biofilm and increase siderophore production

Scott N. Dean, Myung-Chul Chung, and Monique L. van Hoek

School of Systems Biology and National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, VA, USA, 10910 George Mason Circle, Manassas, VA 20110 USA

In many bacteria, the ability to modulate biofilm production relies on specific signaling molecules, either self-produced or made by neighboring microbes within the ecological niche. We analyzed the potential interspecies signaling effect of *Burkholderia* Diffusible Signal Factor (BDSF) on *F. novicida*, a model organism for *F. tularensis* and demonstrated that BDSF both inhibits formation and causes dispersion of *Francisella* biofilm. First, specificity was demonstrated for the cis- vs. the trans- form of BDSF. Using RNA-seq, qRT-PCR, and activity assays we found that BDSF altered the expression of many *F. novicida* genes, including genes involved in biofilm formation, such as chitinases. Using a chitinase inhibitor, the anti-biofilm activity of BDSF was also shown to be chitinase dependent. In addition, BDSF caused an increase in RelA expression and increased levels of (p)ppGpp, leading to decreased biofilm production. These results suggest that exposure of *F. novicida* to BDSF causes biofilm dispersal. Furthermore, BDSF upregulated the genes involved in iron acquisition (figABCD), increasing siderophore production. Thus, this study provides the first evidence for the role and mechanism of DSF-signaling in the genus *Francisella* and suggests interspecies signaling between *Francisella* and other bacteria. Overall, this study suggests that in response to the interspecies DSF signal, *F. novicida* alters its gene expression, which may allow it to adapt to the presence of other species of bacteria in its environment.

S4-12

The role of OXYR in oxidative stress response of *Francisella tularensis* LVS

Marie Honn, Helena Lindgren, Anders Sjöstedt

Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, SE-90185 Umeå, Sweden

Inside hosts, particularly within professional phagocytes, bacteria experience oxidative stress caused by reactive oxygen (ROS), which disrupt the function of vital macromolecules such as proteins and DNA. Protection against ROS is therefore vital for the success of invading pathogens. OxyR is an H_2O_2 activated transcription factor, which in *E. coli* induces the expression of both catalase, which degrades H_2O_2, and the Ferric uptake regulator (Fur), which represses iron-uptake mechanisms. We constructed an in-frame deletion mutant of *oxyR* in *F. tularensis* LVS (Δ*oxyR*) and found that the absence of OxyR left the strain with a reduced catalase activity, leaving it more sensitive to H_2O_2 than the parental strain. The deletion also rendered the strain more sensitive to paraquat and, most strikingly, left the strain highly sensitive to peroxynitrite. Δ*oxyR* was nonetheless able to replicate as efficiently as the parental strain in both the J774 macrophage cell line and in mouse bone marrow derived macrophages. Although replication competent in cell cultures the mutant was unable to replicate to the same extent as the parental strain in mice, where we observed significantly reduced bacterial loads in the livers of mice infected with the *oxyR* deletion mutant compared to mice infected with LVS. These findings show that *F. tularensis*, like *E. coli*, uses the OxyR protein in the defense against oxidative stress and that the defense mechanisms affected by the deletion of this gene are involved in the protection against stress caused by several different types of ROS.

S4-13

Defining the host-derived carbon sources and metabolic pathways required for *Francisella tularensis* intracellular and *in vivo* growth

Lauren Radlinski, Jason Brunton, Shaun Steele, Sharon Taft-Benz and Tom Kawula

Department of Microbiology and Immunology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599, USA

Francisella tularensis is a highly virulent intracellular pathogen capable of invading host cells and replicating to high densities in the cytoplasm. The rapid rate of *F. tularensis* intracellular growth demonstrates that this organism is highly efficient at harvesting and utilizing the necessary resources required to fuel proliferation within a cell, despite the fact that the bacteria must contend with the host metabolic processes and mechanisms for nutrient sequestration. The means by which intracellular pathogens such as *F. tularensis* obtain sufficient carbon for replication within host cells and tissue remain poorly understood. We found that deletion of *glpX*, the gene encoding fructose 1,6-bisphosphatase, interrupts the gluconeogenic pathway and severely diminished growth of *F. tularensis* Schu S4 in both bone marrow derived macrophages and in a murine pulmonary infection model. In contrast, interruption of the glycolytic pathway through the deletion of phosphofructokinase (*pfkA)*, had no effect on *F. tularensis* growth in cells or in mice. Therefore, gluconeogenesis, but not glycolysis, is necessary for intracellular and *in vivo* growth. Because there are a variety of metabolites that feed into the gluconeogenic pathway upstream of *glpX*, we sought to further elucidate the nutritional requirements of *F. tularensis* by generating mutant strains unable to utilize these carbon sources. Specifically, we targeted *gdhA* and *glpA*, enzymes responsible for shuttling amino acids and glycerol into the TCA cycle and the glycolytic/gluconeogenic pathway, respectively. Both mutants showed a marked decrease in intracellular growth. These data suggest that host-derived amino acids and glycerol, but not glucose, are necessary for fueling *F*. *tularensis* carbon metabolism and permitting proliferation in macrophages.

Session 5:

Cell Biology of *Francisella*

Session 5: Cell Biology of *Francisella*

Chairs: Marina Santic
University of Rijeka, Croatia

Helena Lindgren
Umeå University, Sweden

S5-1 Control of a putative anti-virulence factor
Simon L. Dove

S5-2 Guanylate-binding proteins promote *Francisella novicida* lysis in the host cytosol and AIM2 inflammasome activation
Etienne Meunier, Pierre Wallet, Roland F Dreier, Stéphanie Costanzo, Leonie Anton, Sebastian Rühl, Sébastien Dussurgey, Mathias S Dick, Anne Kistner, Mélanie Rigard, Daniel Degrandi, Klaus Pfeffer, Masahiro Yamamoto[5], Petr Broz and Thomas Henry

S5-3 The enigmatic role of the ClpB protein of *Francisella tularensis*
Athar Alam, Igor Golovliov and Anders Sjöstedt

S5-4 Intracellular trafficking of *Francisella tularensis* subsp. *novicida* within *Dictyostelium discoideum*
Valentina Marecic, Mateja Ozanic, Olga Shevchuk, Michael Steinert and Marina Santic

S5-5 *Francisella* and the phagosomal escape – insights to a potential role of the *Francisella* T6SS
Lena Meyer, Jeanette E. Bröms, Martin E. Rottenberg and Anders Sjöstedt

S5-6 Functional NRAMP overcomes the requirement for TLR signaling in *Francisella* infection
Daniel A. Powell and Jeffrey A. Frelinger

S5-7 *Francisella* cell to cell transfer
Shaun Steele, Lauren Radlinski, Sharon Taft-Benz, Jason Brunton and Tom Kawula

S5-1

Control of a putative anti-virulence factor

Simon L. Dove

Division of Infectious Diseases, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA

PmrA is an orphan response regulator in *F. tularensis* that is important for intramacrophage growth. Using both ChIP-Seq and RNA-Seq we identify those regions of the *F. tularensis* chromosome that PmrA associates with and identify those genes that are subject to control by PmrA. Although PmrA can function as both a positive and a negative regulator, we present evidence that the ability of PmrA to repress the expression of a particular target gene is key to its ability to promote intramacrophage growth. Our findings suggest that the importance of PmrA in facilitating intracellular growth is its ability to repress an anti-virulence factor.

S5-2

Guanylate-binding proteins promote *Francisella novicida* lysis in the host cytosol and AIM2 inflammasome activation

Etienne Meunier[2,6], Pierre Wallet[1,6], Roland F Dreier[2], Stéphanie Costanzo[1], Leonie Anton[2], Sebastian Rühl[2], Sébastien Dussurgey[3], Mathias S Dick[2], Anne Kistner[2], Mélanie Rigard[1], Daniel Degrandi[4], Klaus Pfeffer[4], Masahiro Yamamoto[5], Petr Broz[2,7] and Thomas Henry[1,7]

[1] Centre International de recherche en Infectiologie, Inserm U1111, CNRS UMR 5308, Université Claude Bernard Lyon-1, Ecole Normale Supérieure, Lyon, France; [2] Focal Area Infection Biology, Biozentrum, University of Basel, Basel, Switzerland; [3] Structure Fédérative de Recherche Biosciences, UMS344/US8, Inserm, CNRS, Université Claude Bernard Lyon-1, Ecole Normale Supérieure, Lyon, France; [4] Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; [5] Department of Microbiology and Immunology, Osaka University, Osaka, Japan; [6] These authors contributed equally to this work; [7] Co-senior authors Centre International de recherche en Infectiologie, 21 avenue Tony Garnier, 69007 Lyon, France

The AIM2 inflammasome detects double-stranded DNA in the cytosol and induces caspase-1-dependent pyroptosis as well as release of the inflammatory cytokines interleukin 1β (IL-1β) and IL-18. AIM2 is critical for host defense against DNA viruses and bacteria that replicate in the cytosol, such as *Francisella novicida.* The activation of AIM2 by *F. novicida* requires bacteriolysis, yet whether this process is accidental or a host-driven immunological mechanism has remained unclear. By screening nearly 500 interferon-stimulated genes through the use of small interfering RNA, we identified guanylate-binding proteins GBP2 and GBP5 as key activators of AIM2 during infection with *F. novicida*. We confirmed their prominent role *in vitro* and in a mouse model of tularemia. Mechanistically, these two GBPs targeted cytosolic *F. novicida* and promoted bacteriolysis. Thus, in addition to their role in host defense against vacuolar pathogens, GBPs also facilitate the presentation of ligands by directly attacking cytosolic bacteria.

S5-3

The enigmatic role of the ClpB protein of F*rancisella tularensis*

Athar Alam, Igor Golovliov and Anders Sjöstedt

Department of Clinical Microbiology, Umeå University, Umeå, Sweden

It has been previously reported that the *clpB* mutant (Δ*clpB*) of the *Francisella tularensis* SCHU S4 strain is highly attenuated and displays better protective efficacy than the human vaccine strain LVS in mice. In view of the interesting phenotype of the mutant, it is of much interest to specifically identify the role of ClpB and the mechanisms of its attenuation. It belongs to the AAA+ chaperon family, but its exact role in *F. tularensis* is enigmatic. To elucidate the role of the SCHU S4 ClpB protein, we characterized phenotype of the Δ*clpB* mutant in a variety of assays. Previously, the phenotype of the corresponding LVS mutant has been characterized and for comparison, we also included it in the study. In view of its known function as a chaperone, we postulated that it would have a deviant phenotype in response to specific stress stimuli. Indeed the mutants showed very poor survival in vitro in response to both heat shock and low pH but not to H_2O_2. Like the parental strains, both mutants escaped the phagosome and showed effective replication in J774A.1 and bone marrow macrophages, however, cytopathogenic responses were distinctly delayed. Moreover, each of the mutants failed to suppress the LPS-induced TNF-α expression, in contrast to their parental strains. In addition, we found that the secretion of T6SS-mediated effectors in the mutants was perturbed. Furthermore, also replication *in vivo* was followed and it was observed that LVS Δ*clpB* was cleared much faster than LVS from all the organs investigated, in contrast, the Schu Δ*clpB* mutant, despite its marked attenuation, grew to high numbers and persisted longer than LVS. Further studies are in progress to elucidate the mechanisms underlying the enigmatic attenuation of Δ*clpB*.

S5-4

Intracellular trafficking of *Francisella tularensis* subsp. *novicida* within *Dictyostelium discoideum*

Valentina Marecic[1], Mateja Ozanic[1], Olga Shevchuk[2], Michael Steinert[2] and Marina Santic[1]

[1] Department of Microbiology and Parasitology, Medical Faculty, University of Rijeka, Croatia; [2] Institut für Mikrobiologie, Technische Universität Braunschweig, Braunschweig, Germany

Francisella tularensis is a gram-negative bacterium able to replicate in mammalian, arthropod and amoeba cells. *Dictyostelium discoideum* is a soil amoeba that has been used to study virulence factors for many bacterial pathogens, including *Legionella pneumophila*, *Mycobacterium spp*., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. It has been shown that *F. tularensis* is able to survive and replicate in *Acanthamoeba castellanii* and *Hartmanella vermiformis*. Since there are limited opportunities to study intracellular life cycle of *Francisella* in amoebae cells we used *D. discoideum* to explore the intracellular trafficking of *F. novicida* in these cells. First, we show that growth of *Francisella* is depended on the presence of *D. discoideum* in the medium. *F. novicida* replicates exponentially within *D. discoideum* cells and is fatal to the cells a dose-dependent manner, while the *iglC* mutant is severely defective. Using electron and fluorescence microscopy-based phagosome integrity assays, we show that bacterium does not escape into the cytosol of *D. discouidem* but it replicates in the membrane-bound vacuoles. Endocytic trafficking studies of *F. novicida* within *D. discoideum* show co-localization of the bacterial phagosome with *D. discoideum* vatA but not with vacuolin. The data show that *Francisella*-containing vacuole harboring the *iglC* mutant acquire vacuolin, indicating merging with the lysosomal compartments. Our data indicate crucial differences in the intracellular trafficking of *F. novicida* within mammalian and *D. discoideum* cells.

S5-5

Francisella and the phagosomal escape – insights to a potential role of the *Francisella* T6SS

Lena Meyer[1], Jeanette E. Bröms[1], Martin E. Rottenberg[2] and Anders Sjöstedt[1*]

[1] Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, SE-90185 Umeå; [2] Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm SE-17177, Sweden; [*] Correspondence to: anders.sjöstedt@umu.se

Mutants within the *Francisella* Pathogenicity Island (FPI) have been found to be defective for phagosomal escape, intracellular growth and for activation of the inflammasome. Interestingly, the FPI, which is present in two copies in the laboratory strain LVS, encodes a functional type VI secretion system (T6SS) which seems to be different from other described secretion machineries[1]. However, in the past it has been difficult to assess the role of individual FPI proteins for the intracellular life cycle of *Francisella,* as mutants deficient for phagosomal escape never reach the growth-permissive cytosol. Therefore the importance of corresponding proteins for the escape step during infection has not been addressed. We were able to approach this issue using a microinjection technique that allowed us to directly inject FPI mutants with distinct phenotypes (*e.g*. Δ*iglC*, Δ*iglG*, Δ*pdpE*) into the cytosol of different host cells (*e.g.* J774 cells, BMMs, HeLa cells)[2,3]. To our surprise we found that mutants which are unable to escape the phagosomal compartment (*e.g.* Δ*iglC*), replicated after being microinjected into the cytosol and thus, corresponding proteins may be exclusively essential for the phagosomal escape. Strikingly, among the mutants tested we did not identify any that were deficient for replication upon injection implying that corresponding proteins are not essential for intracellular replication only. However, the parental strain LVS showed markedly less replication in some cell types which indicates that the optimal adaptation to the conditions in the phagosome might be a prerequisite for normal intracellular replication.

S5-6

Functional NRAMP overcomes the requirement for TLR signaling in *Francisella* infection

Daniel A. Powell and Jeffrey A. Frelinger

University of Arizona, Department of Immunobiology, 1656 E. Mabel Street Tucson, Arizona 85719, USA

NRAMP (Natural Resistance Associated Macrophage Protein, Slc11a1) is a membrane-associated transporter of divalent metal ions. Murine NRAMP has two known alleles, a functional NRAMP^{Gly169}, which is found in DBA2/J, NOD, and 129p3/J and related mouse strains, and a non-functional NRAMP^{Asp169}, that is found in C57BL/6 (B6) and BALB/c mice. B6 mice congenic for NRAMP^{Gly169} (B6-*NRAMP^{G169}*) show a marked resistance to the intracellular pathogens *Salmonella, Leishmania,* and *Mycobacterium tuberculosis*. In this work we examined the pathogenesis of *Francisella* in B6-*NRAMP^{G169}* mice. Bone marrow derived macrophages (BMDM) from either B6-*NRAMP^{G169}* or B6 mice showed similar levels of *Francisella* invasion at 1 hour post infection (hpi). Interestingly at 16 hpi while the LVS burden in B6 macrophages had increased roughly 100 fold, the B6-*NRAMP^{G169}* burden had decreased 10 fold. When challenged by the intranasal (i.n.) route B6-*NRAMP^{G169}* mice showed no weight loss compared to their starting weight. B6 mice lost significant amounts (~15%) of weight upon challenge. Additionally, organ burdens were examined between B6-*NRAMP^{G169}* and B6 mice. Three days after infection all B6-*NRAMP^{G169}* mice had no detectable *Francisella* in the lung, liver or spleen. B6 mice had burdens approaching 1x10^{6} CFU in all three organs. To further examine the degree of resistance imparted by NRAMP^{Gly169} expression we further challenged mice deficient in TLR2, TLR4, and TLR9, expressing NRAMP^{Gly169} (B6-*NRAMP^{G169} TLR2/4/9$^{-/-}$*). While TLR4 has no effect on *Francisella* pathogenesis TLR2 has been shown to be crucial in B6 mice to surviving *Francisella* infection. Surprisingly, B6-*NRAMP^{G169} TLR2/4/9$^{-/-}$* mice showed no notable weight loss upon i.n. challenge. Plating of tissue homogenate showed 80% of B6-*NRAMP^{G169} TLR2/4/9$^{-/-}$* mice showed no detectable *Francisella*. The mice that did show bacteria were still almost 100 fold less than B6 wild type mice. These data taken together serve to highlight that functional NRAMP^{Gly169} can overcome a lack of TLR2/9. Thus NRAMP is a critical player in murine resistance to *Francisella* infection.

S5-7

Francisella cell to cell transfer

Shaun Steele, Lauren Radlinski, Sharon Taft-Benz, Jason Brunton, Tom Kawula

University of North Carolina, Chapel Hill, NC 27599, USA

Macrophages are myeloid-derived phagocytic cells and one of the first immune cell types to respond to microbial infections. However, a number of bacterial intracellular pathogens are resistant to the antimicrobial activities of macrophages and can actively grow within these cells. Macrophages also have other immune surveillance roles within the host that include the acquisition of cytosolic components from multiple types of host cells. We hypothesized that intracellular pathogens that can replicate within macrophages exploit cytosolic transfer to facilitate bacterial spread. Here we show that viable *Francisella tularensis* bacteria transfer from infected cells to uninfected macrophages along with other cytosolic material through a transient, contact dependent mechanism. Bacterial transfer occurs when the host cells exchange plasma membrane proteins and cytosol via a trogocytosis related process that leaves both donor and recipient cells intact and viable. Trogocytosis was strongly associated with infection in a mouse infection model, suggesting that direct bacterial transfer occurs by this process *in vivo.*

Session 6:

Poster session B

Session 6: Poster session B

S6-1 Using *Dictyostelium* to dissect host-pathogen interactions in *Francisella* infection
Yannick P Brenz , Elisabeth O Lampe , Lydia Herrmann , Urska Repnik , Hanne C Winther-Larsen , Monica Hagedorn

S6-2 Intracellular trafficking of *pilo* mutant within human macrophgaes and pathogenesis of tularemia in BALB/C mice
Mateja Ozanic, Valentina Marecic, Yousef Abu Kwaik and Marina Santic

S6-3 A ciprofloxacin resistant strain of *Francisella tularensis* is highly attenuated in murine models of tularemia
Joel Bozue, Todd Kijek, Ronald Toothman, Christopher Cote, Taylor Chance, Sherry Mou, Nicolas Vietri, and Patricia Worsham

S6-4 Targeting the host response to treat tularemia
RV D'Elia, TR Laws, S Vautier, M Travis, ED Williamson and GC Clark

S6-5 A putative carboxypeptidase is required for *Francisella tularensis* virulence
Sherry Mou, Todd Kijek, Joel Bozue, and Patricia Worsham

S6-6 Zebrafish as vaccine and infection model for *Francisella noatunensis* subsp. *orientalis*
Lagos L., Tandberg J., Winther-Larsen H.

S6-7 Tolc-dependent modulation of host cell death in *F. novicida*
Erik J. Kopping and David G. Thanassi

S6-8 Development of *in vitro* infection of mouse bmdc with *F. tularensis*
V. Hostýnková, I.Fabrik, M. Link, J. Stulík

S6-9 Determining important regulators of immunity to the bacterium *Francisella tularensis*
J. Casulli, R. D'Elia, T. Hussell, E.D. Williamson, G. Clark and M. Travis

S6-10 Deciphering the interaction of *Francisella novicida* with pattern recognition receptors
Camille Robert, Alexiane Decout, Alain Charbit, Thomas Henry, Alain Vercellone and Jérôme Nigou

S6-11 Early cytokine response to virulent and avirulent strains *Francisella tularensis*
Sorokin V.M., Pavlovich N.V., Onopriyenko N.N., Pisanov R.V., Prozorova L.A.

S6-12 GM-CSF has disparate roles in the host response to intradermal and intranasal *Francisella tularensis* LVS infection
Sherry L. Kurtz, Catharine M. Bosio, and Karen L. Elkins

S6-13 The first cells infected by *Francisella* vary by route of entry and shape the immune response and the disease outcome
Jeffrey Frelinger, Daniel A. Powell and Lydia Roberts

S6-14 Limited response of effector and lung-resident memory t cells prevents protection against virulent *Francisella tularensis* pulmonary challenge
Liat Bar-On, Hila Cohen, Uri Elia, Erez Bar-Haim, Shahar Rotem and Ofer Cohen

S6-15 Understanding *Francisella* infection *in vivo* using non-invasive optical imaging
C. A. Hall, S. V. Harding, H.S. Atkins, R. W. Titball

S6-16 Vaccine-mediated mechanisms controlling growth of *Francisella tularensis* in human peripheral blood mononuclear cells
Kjell Eneslätt, Igor Golovliov and Anders Sjöstedt

S6-17 see S5-3

S6-18 see S5-4

S6-19 see S5-5

S6-20 see S5-6

S6-21 see S7-4

S6-22 see S7-8

S6-23 see S7-9

S6-1

Using *Dictyostelium* to dissect host-pathogen interactions in *Francisella* infection

Yannick P Brenz[1], Elisabeth O Lampe[2,3], Lydia Herrmann[1], Urska Repnik[4], Hanne C Winther-Larsen[2,3], Monica Hagedorn[1]

[1] Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; [2] Center of Integrative Microbial Evolution (CIME); [3] Department of Pharmaceutical Biosciences, University of Oslo, PO Box 1068 Blindern, 0316 Oslo, Norway; [4] Department of Biosciences, University of Oslo, Blindernveien, Oslo, Norway

The genus of *Francisella* comprises of Gram-negative, facultative intracellular bacteria that infect various species of both vertebrates and invertebrates. *Francisella* species can be divided into two lineages represented by *F. tularensis* causing potentially fatal tularemia in humans and *F. noatunensis* infecting aquatic animals. We established a novel infection model using *Dictyostelium discoideum* as a surrogate macrophage and the fish pathogen *F. noatunensis subsp. noatunensis* (*F.n.n.*). The *Dictyostelium-F.n.n.* system shares high similarities with the mammalian system demonstrated by comparable phagosomal maturation escape and bacterial growth in the cytosol dependent on the *F.n.n.* virulence factor IglC. With this new model system we want to identify and study bacterial virulence factors as well as host defence mechanisms. In *F. tularensis* infection the role of autophagy is disputed. Immunofluorescence studies on infected *Dictyostelium* cells showed that *F.n.n.* is associated with cellular markers of degradation (Ubiquitin) and the autophagic pathway (p62, Atg8). Real time PCR suggests an increase in autophagy activity indicated by elevated RNA levels of p62 and two atg8 isoforms. When autophagy is impaired (*Dictyostelium* Δ*atg1*) increased intracellular growth of F.n.n. is observed compared to *Dictyostelium* Wt cells at the late stage of infection. Overall, the *Dictyostelium-F.n.n.* system allows us to dissect the interactions between pathogenic *Francisella* and its host cell on the molecular and functional level.

S6-2

Intracellular trafficking of *pilO* mutant within human macrophgaes and pathogenesis of tularemia in BALB/C mice

Mateja Ozanic[1], Valentina Marecic[1], Yousef Abu Kwaik[2] and Marina Santic[1]

[1] Department of Microbiology and Parasitology, Medical Faculty, University of Rijeka, Rijeka, Croatia; [2] Department of Microbiology and Immunology, College of Medicine, University of Louisville, KY, USA

F. tularensis is a gram-negative bacterium able to adhere, invade and replicate in phagocytic and nonphagocytic cells. Many virulence factors of *F. tularensis* have been determined and investigated, including type IV pili (T4P). Previous results showed that type IV pili of *Francisella* subsp. contribute to cell adherence in BMDM, A549 and FL83B cells. However, there is no evidence of the role of pili in intracellular life of *F. tularensis* once inside the human macrophages. In this study transposon insertion mutant of *F. novicida* U112 was used to study the importance of *pilO* gene in virulence of bacteria on *in vitro* and *in vivo* infection models. The results of this study shows that the *pilO* mutant is not able to escape the *Francisella* containing phagosome and consequently, is attenuated for intracellular replication within human macrophages. Further, the *pilO* mutant was attenuated for virulence in BALB/c mice, regardless to the route of infection (intradermal or intratracheal). In contrast to the mice infected with the wt *F. novicida,* no histopathologic changes were obserwed in the lungs, liver and spleen of BALB/c mice inoculated i.t. or i.d. with the *pilO* mutant. We conclude that the *pilO* plays an important role in intracellular replication of *F. novicida*, as well as in the pathogenesis of experimental tularemia in BALB/c mice.

S6-3

A ciprofloxacin resistant strain of *Francisella tularensis* is highly attenuated in murine models of tularemia

Joel Bozue, Todd Kijek, Ronald Toothman, Christopher Cote, Taylor Chance, Sherry Mou, Nicolas Vietri, and Patricia Worsham

The United States Army of Medical Research Institute of Infectious Diseases; Fort Detrick, MD, USA

Francisella tularensis is a gram–negative facultative intracellular bacterial pathogen that can infect many mammalian species, including humans. *F. tularensis* has two subspecies, subspecies *tularensis* (type A) and subspecies *holarctia* (type B). Both subspecies are infectious for humans, but type A strains are typically more lethal. Because of its ability to case a lethal infection, its low infectious dose, and aerosolizable nature, *F. tularensis* subspecies *tularensis* is considered a potential bioterrorism agent. Although not FDA approved, fluoroquinolones have been used to treat *F. tularensis holarctica* infections. Because of its in vitro efficacy against *F. tularensis* subspecies *tularensis*, ciprofloxacin is one of the antibiotics recommended for post exposure prophylaxis. In order to identify therapeutics that will be efficacious against infections caused by drug resistant select-agents, we sought to characterize an existing ciprofloxacin resistant mutant of *F. tularensis* by determining the mechanism of acquired antibiotic resistance and its phenotypic characteristics, including virulence. Selection of a ciprofloxacin resistant mutant of the Schu4 strain has been previously described and approved by the CDC. We further characterized this mutant for differences in growth in medium and macrophage-like cells as well as virulence in various murine models of tularemia. Initial characterization of the mutant showed differences in growth depending on the type and composition of the media. We determined that the presence of additional thiamine greatly enhanced growth of the mutant strain. In addition, the mutant demonstrated a defect in recovery when incubated with macrophage-like cells, in contrast to the robust intracellular growth observed with the wild-type Schu4 strain. Based upon these results, the mutant was tested for the ability to cause infection in mouse models of tularemia. The murine LD_{50} for the wild-type strain by intranasal and intradermal challenge is 1-2 CFU. In contrast, the LD_{50} for the ciprofloxacin resistant mutant by these challenge methods was 14,000 and >49,000 CFU, respectively. Virulence was further assessed between strains by examining differences in the dissemination from the lung to the spleen following pneumonic challenge. Understanding the underlying mechanisms of this attenuation is important from a defense perspective, allowing us to determine what effect the acquisition of ciprofloxacin resistance has on virulence to better predict the outcome of a deliberate manipulation of this pathogen.

S6-4

Targeting the host response to treat tularemia

RV D'Elia[1], TR Laws[1], S Vautier[2], M Travis[2], ED Williamson[1] and GC Clark[1]

[1] Dstl, Porton Down, Biomedical Sciences Department, Microbiology Group, Immunology, Bldg 7a, Salisbury. SP4 0JQ. UK; [2] Manchester Collaborative Centre for Inflammation Research (MCCIR), University of Manchester, Oxford Road, Manchester. M13 9PT. UK

Francisella tularensis can cause a variety of different diseases dependent on the route of infection. Inhalational exposure represents the most likely route of attack if this bacterium was to be used as a BioThreat agent. The immune response within the lung following inhalational mouse models of infection is temporal; demonstrating an initial lack of pro-inflammatory cytokine response, followed by an exponential increase (termed Cytokine Storm) 1-2 days prior to death. Both of these aspects contribute to the pathogenesis of the disease and therefore represent potential targets for the generation of post-exposure therapeutics. Over the last few years our laboratory has focused on understanding the overactive immune response and explored the use of anti-inflammatory compounds (either alone or in combination with antimicrobials) to potentially increase survival and/or extend time to death. We have demonstrated proof of principle that the use of a host-targeted therapy (i.e. anti-HMGB1 antibodies), in combination with levofloxacin during a *F. tularensis* Schu S4 infection can increase survival and widen the window of opportunity for traditional antibiotic treatments. Further, using immuno-histochemistry, the potential role of macrophages and regulatory T cells in the pathogenesis of the disease was also identified. Recently we have explored the role of regulatory T cells following infection with *F. tularensis*, and the consequences of their manipulation in the context of immune dysregulation. Using DEREG mice, which specifically deplete FOXP3 regulatory T cells through the administration of diphtheria toxin, we have shown that bacterial burden in the liver and spleen is significantly reduced compared to wild type controls during an infection with *F. tularensis* live vaccine strain. Our ongoing research on the host immune response looks to establish the key immune cells/molecules during infection in order to improve the likelihood of identifying a novel (host-targeting) post-exposure therapeutic that can treat tularemia and/or increase the efficacy of existing antimicrobials.

S6-5

A putative carboxypeptidase is required for *Francisella tularensis* virulence

Sherry Mou, Todd Kijek, Joel Bozue, and Patricia Worsham

The United States Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, USA

Francisella tularensis is a zoonotic intracellular pathogen and the causative agent of tularemia. After infection of a macrophage, the organism subsequently escapes from the phagosome, replicates to high density in the cytosol and triggers phagocyte death and lysis. Unfortunately, many of the bacterial factors required for these aspects of virulence have not yet been identified. Here we describe the isolation and characterization of a *Francisella tularensis subsp. tularensis* strain Schu S4 mutant that lacks a functional FTT1029 gene. The genome annotation of *F. tularensis* predicts that the FTT1029 locus functions as a D-alanine carboxypeptidase. Our data demonstrated that this mutant was defective for replication in murine J744.A1 cells, A549 cells and also exhibited reduced growth in defined media. When virulence was assessed by intranasal infection of BALB/c mice, the mutant appeared highly attenuated (mutant LD50= > 300 CFU vs the parental strain LD50~1.0 CFU). Complementation studies using the native gene provided in *trans* resulted in 100% restoration of the wild-type phenotypes. Our data suggests that this putative carboxypeptidase plays an important role in the virulence of *F. tularensis* Schu S4.

S6-6

Zebrafish as vaccine and infection model for *Francisella noatunensis* subsp*. orientalis*

Lagos L., Tandberg J., Winther-Larsen H.

Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, PO Box 1068 Blindern, 0316 Oslo, Norway

Francisella consist of highly virulent bacterial species that are able to cause infection in a variety of vertebrates and invertebrates including humans: *F. tularensis* and *F. philomiragia*, as well as in fish: *F. noatunensis*. *F. noatunensis* consists of two subspecies, which appear to be adapted to different host temperatures. *F. noatunensis* subsp. *orientalis* (*Fno*) causes disease in "warm-water" fish, like tilapia, while *F. noatunensis* subsp. *noatunensis* (*Fnn*) causes disease in fish living in colder waters, like cod. In tilapia, fish infected with *Fno* present nonspecific clinical signs, such as erratic swimming, anorexia, exophthalmia and high mortality (50 to 60%). The cultivation of *Fno* has shown the secretion of outer membrane vesicles (OMVs). The production of OMVs is a common trait for several bacterial species of both environmental and pathogenic nature. OMV secretion is associated with a variety of traits and in pathogenesis it is allowing discharge of virulence factors during infections as a part of the host pathogen interaction. Moreover, isolated OMVs have been used successfully as vaccines against diseases caused by other bacteria and have shown potential as a vaccine against francisellosis caused by *Fnn.* A *Fno* – adult Zebrafish model has previously been established and in our lab we established a Zebrafish embryo model for *Fno*. Both adult and embryo Zebrafish models are found suitable for studies of *Fno* pathogenesis. We investigated the virulence and pathogenicity of *Fno* and its OMVs in an adult Zebrafish infection model. We report on the Zebrafish immune response to *Fno* and *Fno* produced OMV by measuring the transcriptional profile of important immune effectors like proinflammatory cytokines (TNF-α, IL-1β and IL-6), IL-12, IFNγ, IL-10 and different cell markers (IgM, BLIMP, CD40, Mpeg, Mpx). Additionally, cell from kidney were isolated and by the use of flow cytometry we were able to identify the cellular subpopulation involve in the infection of *Fno*. Our results show Zebrafish as a relevant and comparative model system to study host pathogen interaction during *Fno* infection. This model may be applicable to other fish species of importance in the aquaculture and will enable further studies of *Francisella* and thereby the potential development of a vaccine or treatment plan against francisellosis.

S6-7

TolC-dependent modulation of host cell death in *F. novicida*

Erik J. Kopping and David G. Thanassi

Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY, USA
295 Centers for Molecular Medicine Building, Center for Infectious Diseases, Stony Brook University, Stony Brook, NY 11794-5120, USA

One hallmark of *Francisella* infection is the bacterium's ability to replicate within the cytosol of host cells. To facilitate this intracellular growth, *Francisella* dampens host cell innate immune responses and delays activation of host cell death pathways. Recently, our lab characterized the ability of the *F. tularensis* Live Vaccine Strain (LVS) to delay the induction of apoptosis in infected macrophages to preserve its replicative niche, as mediated by the channel protein TolC. TolC is the outer membrane component of tripartite export systems including multidrug efflux pumps and the type I secretion system (T1SS). T1SS function in the export of bacterial toxins and other virulence factors. As *F. novicida* is closely related to both the LVS and fully virulent *F. tularensis* strains, it serves as a common model for *Francisella* infection. Utilizing the defined *F. novicida* U112 transposon mutant library, we hypothesized that *F. novicida* could serve as a useful model to understand TolC-mediated drug efflux and modulation of the macrophage response to *Francisella* infection. We report that similar to the LVS, *F. novicida* TolC serves an important role in mediating multidrug resistance and modulating host cell death in response to infection. Infection of primary murine macrophages with a *F. novicida tolC* mutant resulted in increased macrophage cytotoxicity, correlating with a loss of the intracellular replicative niche. Additionally, we identified another T1SS component, EmrA1, which also functions in resistance to antimicrobial compounds and modulation of host cell death during *F. novicida* infection. We found that infection of macrophages with either *F. novicida emrA1* or *tolC* mutants resulted in premature caspase-3 activation. However, macrophages from *caspase- 1/11*$^{-/-}$-deficient mice did not exhibit premature cell death in response to infection with the *F. novicida* mutants. This suggests that the premature host cell death elicited during both the *F. novicida tolC* mutant and *emrA1* mutant infection is largely dependent on inflammasome activation and not due to the intrinsic apoptotic pathway, in contrast to what was observed for infection with the LVS *ΔtolC* mutant. Our findings demonstrate that TolC function is conserved among different species of *Francisella*, but also highlight differences in host cell death responses to infection by *F. tularensis* versus *F. novicida*. These altered host cell responses may, in part, underlie the decreased virulence of *F. novicida* in humans.

S6-8

Development of *in vitro* infection of mouse bmdc with *F. tularensis*

V. Hostýnková, I.Fabrik, M. Link, J. Stulík

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, Třebešská 1575, 500 01 Hradec Králové, Czech Republic

Francisella tularensis is a highly virulent Gram-negative bacterium causing a zoonotic disease tularemia. It is an intracellular pathogen invading many types of cells, where it survives, proliferates and finally kills the host cells to spread further. The bacterium is therefore able to manipulate and undermine the host defense mechanisms. It enters preferentially phagocytic cells including macrophages, dendritic cells (DCs) and neutrophils. DCs are important immune cells with unique properties that influence the innate and adaptive immune responses. DCs are efficient antigen-presenting cells that are able to activate naive T cells, both *in vivo* and *in vitro*. The bacterium enters and multiplies inside DCs *in vitro*. Recently described results indicate that *Francisella tularensis* signals through TLR-2 for the induction of inflammatory cytokines. Recognition of microbial products by TLR leads to activation of NF-κB, production of cytokines and the induction of costimulatory molecules. *Francisella* colocalizes with the lysosomal marker LAMP-1 early after DCs entry, denoting that it resides within *Francisella*-containing phagosome. Thereafter, majority of bacteria escape to the cytosol of murine DCs where it replicates. We aim to study the interaction of *Francisella tularensis* with DCs, in view of the DC activation and antigen presentation. We present the establishment of *in vitro* model of infection on the mouse bone marrow-derived DC. DCs were obtained from GM-CSF cultures. For *Francisella tularensis* infection, the bacterium dose (MOI) and duration of infection dependence was evaluated with respect to DC viability and maturation. DCs maturation was assessed by CD86, CD80, CD40, and MHCII cell surface markers using flow cytometry. The success of infection was confirmed via fluorescence microscopy and proliferation assay. The MOI of 1-5 appeared optimal for 24h infection. For verification of possible inhibition effect of *Francisella* to production of cytokines was evaluated its production in time dependent and dose dependent manner.

S6-9

Determining important regulators of immunity to the bacterium *Francisella tularensis*

J. Casulli[1], R. D'Elia[2], T. Hussell[1], E.D. Williamson[2], G. Clark[2] and M. Travis[1]

[1] MCCIR, University of Manchester, UK; [2] Dstl Porton Down, Salisbury, UK Manchester Collaborative Centre for Inflammation Research, University of Manchester, 2nd floor, Core Technology Facility, Oxford Road, Manchester, M13 9PT, UK

The lungs have evolved to maintain their vital function of gaseous exchange, while interacting with a myriad of airborne microorganisms. Pulmonary immune homeostasis is important in balancing inappropriate immune responses against innocuous antigens and protective responses against harmful pathogens. Negative immunoregulatory receptors in the lung play an important role both in healthy conditions and during disease, with alveolar macrophages playing a major part in maintaining immune homeostasis. *Francisella tularensis* is a Gram-negative intracellular bacterium characterised by a dampened immune response during early pneumonic infection. Increased virulence via the respiratory route has led to concern about its potential use as a biothreat agent. As *F. tularensis* has been shown to preferentially infect alveolar macrophages during early stages of infection, we aim to investigate whether negative immune regulators in the lung play a role in dampening early responses following *F. tularensis* live vaccine strain (LVS) infection. *In vitro* macrophage models will allow analysis of basic cellular responses to LVS infection both at the mRNA and protein level. Host responses in negative regulator gene knockout mice infected intranasally with LVS will also be characterised. Host weights and organ bacterial burdens will be measured at various days during infection both early and late to determine how the knockout mice control the course of LVS infection. Flow cytometry of organ homogenates, cytokine analysis and immunofluorescence will provide further detail into how the negative immune regulators in the lung can impact the immune response towards LVS. Following from the identification of important regulatory pathways, we will assess the outcome of therapeutic blockade during LVS infection. Understanding how the bacterium is able to achieve early immune suppression could have major implications for the development of treatments and vaccines for *F. tularensis* and possibly other respiratory pathogens. As the lung has the exceptional ability to maintain homeostasis between innocuous and harmful pathogens, the negative regulatory pathways seem an extremely likely target for evasive pathogens such as *F. tularensis*. Therefore investigating novel interactions, as well as those already seen for other respiratory pathogens, should be the next step forward in research on *F. tularensis*.

S6-10

Deciphering the interaction of *Francisella novicida* with pattern recognition receptors

Camille Robert[1,2], Alexiane Decout[1,2], Alain Charbit[3,4], Thomas Henry[5,6], Alain Vercellone[1,2] and Jérôme Nigou[1,2]

[1] CNRS, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France ; [2] Université de Toulouse, Université Paul Sabatier, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France; [3] Université Paris Descartes, Sorbonne Paris Cité, Bâtiment Leriche, France; [4] INSERM U1151 - CNRS UMR 8253, Institut Necker-Enfants Malades, Paris, France; [5] Centre international de recherche en infectiologie, Université de Lyon, Lyon, France; [6] Bacterial Pathogenesis and Innate Immunity Laboratory, INSERM U851 "Immunity, Infection and Vaccination", Lyon, France

Virulence strategy of *Francisella tularensis* relies in part on its ability to modulate innate immune response, which is based on the detection of molecular signatures of microbes *via* a limited number of Pattern Recognition Receptors (PRRs). For instance, *F. tularensis* produces an inert LPS that does not induce Toll-like Receptor 4 (TLR4) signaling or represses bacterial lipoprotein production, thereby dampening TLR2 activation. However, the bacterial ligands, host cell receptors and signaling pathways modulated during innate immune recognition of the pathogen remain largely unknown. We have thus undertaken the systematic analysis of bacteria interaction with the different families of PRRs, including TLRs, C-type lectin-like receptors (CLRs) and NOD-like receptors (RLRs) using *Francisella novicida* as a surrogate species.

S6-11

Early cytokine response to virulent and avirulent strains *Francisella tularensis*

Sorokin V. M., Pavlovich N. V., Onopriyenko N. N., Pisanov R.V ., Prozorova L. A.

Rostov-on-Don Research Institute for Plague Control, Rostov-on-Don, str. M. Gorkogo 117, 344002, Russia

Previously, we have shown that the implementation of the biological properties of *F. tularensis* LPS *in vivo* takes place only when it is presented by live bacterial cells (Onopriyenko 2003). Therefore, the comparative RT-PCR study on cytokine-inducing activity of virulent *F. tularensis* bacteria of three main subtypes and their isogenic avirulent LPS-deficient variants was conducted to determine the influence of the *F. tularensis* LPS structure on the induction of cytokine response of white mice PMN . As observed, all the studied avirulent strains demonstrated similar cytokine induction profiles with slight variations depending, apparently from individual characteristics of the strains. In particular, they caused early induction of proinflammatory cytokines TNF-α, IL1-β, and immunosuppression factor TGF- β. The R-LPS strains 543 (subsp. *mediasiatica*) and 503 (subsp. *holarctica*) were characterised by decreasing over time the level of mRNA synthesis of TNF-α, IL1-β and TGF-β. At the same time, strain 261 (subsp. *tularensis*) (R-LPS), along with an early induction of TNF-α, caused only a late induction of IL1-β and IFN- γ. This is consistent with the results obtained by us using ELISA, that the cytokine response of immunocompetent white mice cells to F.tularensis 261 differs from the strains of two other subspecies: it is characterized by early activation of TNF-α by both virulent and and LPS - deficient variants. On the contrary, complete absence of induction TNF-α and IFN-γ with pronounced induction of IL1-β and immunosuppressive factor TGF- β by pathogenic strains subspecies holarctica and mediasiatica was demonstrated. Therefore, avirulent *F. tularensis* LPS-deficient mutants activate the innate immune system, which apparently leads eventually to the elimination of microbes from the host cells. In contrast, the virulent bacteria modified cytokine response (lack of some basic pro-inflammatory cytokines in the early stages of infection) and evade host “recognition” system. It is possible, that this phenomenon is related with immunosuppression factor TGF- β and TNF-modifying ability of bacteria. Thus, it was established that the cytokine response of white mice PMN to virulent strains of *F. tularensis* is significantly different from the response to avirulent bacteria. In particular, they are characterized by the absence of early activation some key inflammatory cytokines and induction of immunosuppressor TGF- β. These results demonstrate that the structure of *F. tularensis* LPS makes an important contribution to the host-parasite interaction and possibly defines “evasion” of virulent bacteria with S-LPS from the innate immune system of the host.

S6-12

GM-CSF has disparate roles in the host response to intradermal and intranasal *Francisella tularensis* LVS infection

Sherry L. Kurtz[1], Catharine M. Bosio[2] and Karen L. Elkins[1]

[1] U.S. Food and Drug Administration, Center for Biologics Evaluation and Research, 10903 New Hampshire Ave, Silver Spring, MD, USA 20903; [2] Rocky Mountain Laboratories, NIAID, Hamilton, MT, USA

Francisella tularensis LVS, a human vaccine, also serves as a useful model organism for studying mechanisms of vaccine induced protection. We previously combined *in vitro* and *in vivo* approaches to screen for immune effectors that are associated with the degree of protection against LVS, and among the effectors found was GM-CSF. GM-CSF is a pleiotropic cytokine that plays a major role in the host immune system in the survival and proliferation of myeloid lineage cells. In particular, GM-CSF is integral in the development of alveolar macrophages and the localization of those cells to the lungs. GM-CSF also has a demonstrated role in primary resistance to several intracellular pathogens and is produced by T cells. *Francisella* is an intracellular pathogen that infects the lungs, and alveolar macrophages are a primary cellular host for LVS. Given this and the link between GM-CSF, lungs and alveolar macrophages, and GM-CSF as a correlate, we explored the role of GM-CSF in primary and adaptive immunity to LVS. Interestingly, GM-CSF has route-dependent roles in primary resistance to LVS. When wild type and GM-CSF deficient (GM-CSF KO) mice were given an intradermal LVS infection, the GM-CSF KO mice were slightly more sensitive to infection, and exhibited a decreased LD_{50} of ~0.5 $\log_{10}$. However, the opposite was true when the mice were infected intranasally. GM-CSF KO mice were considerably more resistant to i.n. LVS infection and had an increased LD_{50} of more than 3 logs. Given the substantial increase in resistance, these mice were also challenged with the virulent SchuS4 strain. Initial findings suggest that the GM-CSF KO mice may have a survival advantage even against this fully virulent pathogen. We further investigated the role of GM-CSF in adaptive immunity. GM-CSF KO mice vaccinated by either the intradermal or intranasal route survived a lethal intraperitoneal or intranasal challenge. T cells from either vaccine group controlled intracellular LVS growth, suggesting that they are primed and functional. Notably, the GM-CSF KO mice had dramatic increases in antibodies, in IgM, IgG, and all of the IgG subclasses, which may also contribute to survival of secondary lethal challenge. Taken together, our data demonstrates that GM-CSF has an active, but complex, role in the host response to LVS. Further studies are currently underway to elucidate its roles, in alveolar macrophages, and how this may influence host immunity to LVS.

S6-13

The first cells infected by *Francisella* vary by route of entry and shape the immune response and the disease outcome

Jeffrey Frelinger[1], Daniel A. Powell[1] and Lydia Roberts[2]

[1] Department of Immunobiology, University of Arizona, Tucson, AZ; [2] Rocky Mountain Labs, NIAID, Hamilton, MT, USA

It has been known for many years that the minimum infectious and lethal dose of *Francisella* varies greatly depending on the route of infection. This is true for both the highly virulent SchuS4 strain and for the vaccine strain, LVS, in both man and mouse. The reason for this difference has remained elusive. It seems likely that the cells initially infected by *Francisella* upon entry into the host are the source of the first innate response. If the identity of the cells infected depended on the route of entry, and they differed in their cytokine profiles, it would explain the differences in immune responses, and the subsequent disease course. Our lab has carefully defined the immune response to Francisella LVS administered either intradermally or intranasally. These two routes of infection produced distinctive immune responses in the lung. Intranasal infection produced a mixed IL-17 and IFN-γ response, while intradermal infection resulted in an INF-γ response, with no detectable IL-17. This is particularly interesting because previous work has shown that IL-17 knockout mice are susceptible to LVS infection in the lung. What could cause this? We have shown that the cells infected soon after LVS intranasal inoculation are alveolar macrophages. Intradermal infection at the base of the tail results in infection of primarily interstitial macrophages in the lung. Thus, the cells that initially encounter *Francisella* in the lung differ by infection route. Alveolar macrophages and interstitial macrophages have distinct lineages and distinct gene expression profiles. Consistent with this result is the difference in the subsequent immune responses in the lung. In normal mice the expression of Nramp1 (*Slc11a1*) differs between alveolar macrophages monocyte derived macrophages. Nramp1 deficiency in mice has been associated with susceptibility to the intracellular bacteria *Salmonella* and BCG. Since much of the immunology of *Francisella* has been done in B6 mice that lack a functional Nramp1 protein. We showed that B6-Nramp1$^+$ mice (with the *Slc11a1^r* gene from 129 mice) are highly resistant to intranasal infection with LVS. B6-Nramp1$^+$ mice clear the infection within a few days, and produce little adaptive or innate response. In contrast, B6 mice become infected and have a robust innate and adaptive immune response following intranasal infection, as has been documented for many years. Nramp1 function had been previously reported to have no effect in Francisella infection based on a footpad inoculation model. When we repeated the footpad injection, we found that as previously reported, there was no difference in the bacterial load or the innate responses (as measured by cytokine production in the serum) between Nramp1$^+$ and Nramp1$^-$ mice. In contrast, intranasally infected Nramp1$^+$ mice showed essentially no cytokine production in the serum or lung, consistent with a rapid clearing before the induction of any innate response, while Nramp1- mice had a pro-inflammatory cytokine response. Taken together these observations strongly support the idea that early interactions with the immune system differ indifferent locations and that these differences direct the outcome of exposure to *Francisella*.

S6-14

LImited response of effector and lung-resident memory T cells prevents protection against virulent *Francisella tularensis* pulmonary challenge

Liat Bar-On, Hila Cohen, Uri Elia, Erez Bar-Haim, Shahar Rotem and Ofer Cohen

Biochemistry and Molecular genetics department, The Israel Institute for Biological Research, Israel

Vaccination with the Live Vaccine Strain (LVS) provides limited protection against pulmonary challenge with the virulent strain SchuS4, which diminishes significantly over time. Here we show that this limitation can be circumvented by boosting with LVS, which results in complete protection to a high pulmonary SchuS4 challenge. However, this enhanced protection diminishes also over time. To shed light on the mechanisms responsible for the protective memory response we analyzed the *in vivo* host responses in the protected and unprotected mice following challenge. As early as 3-4 days following high SchuS4 challenge, the protected mice exhibited significantly reduced bacterial growth and burden, as well as improved morbidity. We show *in vivo* that lung resident memory T cells (T_{RES}) and effector-memory T cells (T_{EM}) produced cytokine that initiated an “antimicrobial” state within the lung. Moreover, we demonstrate that the immediate response of T_{EM} and lung T_{RES} memory cells correlates with the ability of the memory response to clear pulmonary challenge, suggesting that these cells may play a key role in the memory response to tularemia. This study implies that effective long lasting vaccination against pulmonary challenge with virulent *F. tularensis* may require generation and preservation of high T_{EM} and Lung resident memory T cells.

S6-15

Understanding *Francisella* infection *in vivo* using non-invasive optical imaging

C. A. Hall[1], S. V. Harding[2], H.S. Atkins[2], R. W. Titball[1]

[1] Bacterial Pathogenesis Research Group, University of Exeter, Exeter, UK;
[2] Biomedical Sciences, Defence Science and Technology Laboratory, Porton Down, UK, A201, Building 7, Dstl, Porton Down, Salisbury, UK

Optical imaging using bioluminescence allows infection to be monitored non-invasively both longitudinally and in real time. Genes encoding luciferases such as *lux* can be engineered into pathogens to enable the generation of light which can be visualized through the tissues of a live animal using a sensitive, charge-coupled device camera. Moreover, sequential imaging of the same animal enables bacterial kinetics and load to be determined without having to sacrifice the animal thus reducing animal usage. We have developed a bioluminescent strain of *Francisella tularensis* subsp*. tularensis* strain SCHU S4 suitable for use with optical imaging modalities. *F. tularensis* SCHU S4 is a highly virulent pathogen with a low aerosol infectious dose (ID_{50} <10 CFU) and, as such, may be considered a potential biothreat agent. The bioluminescent strain, SCHU S4-*lux,* has been used to develop a mouse model of pneumonic tularemia suitable for the study of host-pathogen interactions and evaluation of therapeutics. The shuttle vector pEDL41 encoding the *luxCDABE* operon was introduced into *F. tularensis* SCHU S4 by electroporation. Following *in vitro* characterization of the SCHU S4-*lux*, virulence was determined in both intranasal and aerosol models of infection. For longitudinal pathogenicity studies shaved BALB/c mice were infected by the aerosol route with 50 CFU of *F. tularensis* SCHU S4-*lux* and infection was imaged sequentially using an IVIS Spectrum whole body imaging system. Organs were explanted and imaged post mortem to confirm anatomical location of signal. Bacterial enumeration was conducted to measure bacterial load and determine correlation between total flux from 2D bioluminescent images and CFU numbers. This study has demonstrated that a bioluminescent strain of *F. tularensis* SCHU S4 was equivalent to wild-type SCHU S4 in growth *in vitro*, either intracellular or in liquid culture. Additionally the plasmid bearing the *lux* operon was stably replicated in the absence of antibiotic selective pressure *in vivo*. Moreover, virulence of SCHU S4-*lux* in a mouse inhalational challenge model was equivalent to that of the wild-type SCHU S4. Bioluminescent signal was detectable from 3 days post-challenge, initially in the lymph nodes and spleen. At the humane endpoint animals had strong signals detectable in the lungs, liver, spleen and lymph nodes. Bacterial load in organs was also comparable between *F. tularensis* SCHU S4-*lux* to wild-type SCHU S4. These data demonstrate non-invasive optical imaging and the strain described are suitable for the study of *F. tularensis* pathogenesis *in vivo* and evaluating the efficacy of therapeutic approaches for tularemia.

S6-16

Vaccine-mediated mechanisms controlling growth of *Francisella tularensis* in human peripheral blood mononuclear cells

Kjell Eneslätt, Igor Golovliov, and Anders Sjöstedt

Department of Clinical Microbiology, Umeå University, Umeå, Sweden

Tularemia or vaccination with the live vaccine strain (LVS) of *Francisella tularensis* confers very long-lived cell-mediated immunity and efficacious protection, but the mechanisms behind this immunity have not been identified in detail. To better understand the mechanisms, we established an *in vitro* culture assay to identify how control of infection of *F. tularensis* is effectuated by human cells and hypothesized that the model will mimic the *in vivo* immune mechanisms. Non-adherent peripheral blood mononuclear cells (PBMC) were added to cultures with adherent PBMC infected with the LVS strain or the highly virulent SCHU S4 strain. Intracellular growth of *F. tularensis* was followed for 72 h and secreted and intracellular cytokines were analyzed. PBMC expanded from naïve individuals, *i.e*., those with no record of immunization to *F. tularensis*, generally showed no control of intracellular bacterial growth, whereas PBMC from a majority of *F. tularensis*-immune individuals executed static and sometimes cidal effects on intracellular bacteria. Regardless of infecting strain, the inhibition of intracellular bacterial growth was very similar. Secretion of 11 cytokines was analyzed after 72 h of infection and their correlations with control of bacterial replication were determined. Intracellular cytokine staining demonstrated that CD4+ T cells showed markedly higher expression of IFN-γ, MIP-1β, TNF-α and CD107a, while CD8+ T cells had higher levels of MIP-1β and CD107a in cultures with cells from immune than in those from naïve individuals. The cell culture system serves as an *in vitro* model for detailed characterization of the mechanisms of the human protective immunity to *F. tularensis*.

Session 7:

Host responses to *Francisella* Part A

Session 7, Part A: Host responses to *Francisella,* Part A

Chairs: Karen Elkins
CBER/FDA, USA

Catharine Bosio
NIAID/NIH, USA

S7-1 Non-proteinaceous virulence factors of *Francisella tularensis*
Catharine M. Bosio

S7-2 Characterizing differences between lvs strains and the implications for elicting immunity
B Jones, J Fletcher and C Bosio

S7-3 *Francisella* manipulates host glycosylation pathways and the UPR response in human macrophages
Monique Barel, Anne Harduin-Lepers, Lucie Portier, Marie-Christine Slomianny and Alain Charbit

S7-4 Complement C3 as a determinant of macrophage death during *Francisella* infection
Susan Renee Brock and Michael Parmely

S7-5 Intestinal goblet cells can augment M-cells for entry of oral vaccines
Aimee L. Cunningham, M. Neal Guentzel, Jieh-Juen Yu, Karl E. Klose, Tonyia D. Eaves-Pyles and Bernard P. Arulanandam

S7-1

Non-proteinaceous virulence factors of *Francisella tularensis*

Catharine M. Bosio

Immunity to Pulmonary Pathogens Section, Laboratory of Bacteriology, Rocky Mountain Laboratories, NIAID/NIH, Hamilton, MT, 59840, USA

The primary mechanism of virulence for *Francisella tularensis* is its ability to evade triggering and subsequently suppress inflammatory responses within the first few hours of infection. However, the bacterial components required for evasion and suppression of these responses are largely undefined. Lipids and carbohydrates that decorate the surface of *F. tularensis* are among the first microbial structures encountered by the host cell and, as such, these non-proteinaceous components of *F. tularensis* are critical for rapid modulation of the host response. We will present on overview of the identification of the lipid and carbohydrate species responsible for inhibiting host inflammatory responses and the specific pathways targeted by these bacterial components to ensure successful infection and replication of virulent *F. tularensis* in the host.

S7-2

Characterizing differences between LVS strains and the implications for eliciting immunity

B Jones[1,2], J Fletcher[1], C Bosio[3]

[1] The Interdisciplinary Graduate Program in Genetics, University of Iowa;
[2] Department of Microbiology, Carver College of Medicine, University of Iowa;
[3] Laboratory of Intracellular Parasites, Rocky Mountain Labs, National Institute of Allergy and Infectious Diseases, USA

Francisella tularensis is a highly virulent bacterial pathogen with an extremely low infectious dose (~10 CFU) and high rates of mortality, if left untreated (30-60%). *F. tularensis* has an extensive history as a bioweapon, and there is no vaccine currently licensed. For these reasons the CDC considers *F. tularensis* a Tier 1 Select Agent. The unlicensed Live Vaccine Strain (LVS) provides moderate protection against virulent strains; however, we have recently discovered that various lab stocks differ in their virulence and ability to confer immunity. Genome sequencing of high virulence (RML, LD_{50} ~200 CFU) and low virulence (ATCC, LD_{50} ~9,000 CFU) strains has identified nine differences, of which four are non-synonymous substitutions. One such mutation occurs in the ferrous iron uptake gene *feoB* in RML. While iron is required for cellular function, ferrous iron can participate in the Fenton reaction with H_2O_2, leading to inactivation of essential iron-sulfur cluster enzymes, and DNA damage. Part of the innate immune response involves the oxidative burst in the phagosome and mitochondria-derived ROS in the cytosol. Fully virulent strains of *F. tularensis* are known to be highly resistant to such host defences, and have low levels of intracellular iron. Accordingly, the RML strain was highly resistant to exogenous H_2O_2 *in vitro* relative to the ATCC strain. Overexpression of the ATCC *feoB* allele, but not the RML allele, leads to significantly increased sensitivity to H_2O_2. Furthermore, the RML strain grows poorly under conditions of iron starvation, and an iron-responsive *lacZ* reporter had ~3-fold higher activity in the RML strain relative to ATCC under these conditions. Overexpression of the iron-responsive transcriptional repressor *fur* leads to reduced growth in the RML strain, but not ATCC. These results are consistent with the hypothesis that RML has less intracellular iron, and that this may lead to increased resistance to host-mediated oxidative stress. These results are being used to aid our studies in developing an effective vaccination strategy against virulent *F. tularensis* infection.

S7-3

Francisella manipulates host glycosylation pathways and the UPR response in human macrophages

Monique Barel[1,2], Anne Harduin-Lepers[3,4], Lucie Portier[3,4], Marie-Christine Slomianny[3,4], Alain Charbit[1,2]

[1] Université Paris Descartes, Sorbonne Paris Cité, Paris, France; [2] Institut Necker Enfants-Malades INSERM, U1151, Team 11, Unité de Pathogénie des Infections Systémiques, Paris, France; [3] UMR CNRS 8576, FR 3688, Villeneuve d'Asq, France; [4] Unité de Glycobiologie Structurale et Fonctionnelle, Université Lille Nord de France, Lille1, Villeneuve d'Asq, France

F. tularensis must obtain nutrients to sustain its replication in infected host cells. We previously demonstrated that the eukaryotic glutamine transporter SLC1A5 was up regulated upon *F. tularensis* LVS infection. LVS infection also induced the deglycosylation of SLC1A5. Presence of the bacteria in the cytoplasmic compartment was necessary to sustain this activity, as an *iglC* mutant unable to egress from the phagosome did not promote this deglycosylation. Protein deglycosylation was apparently specific of *Francisella* infection but not uniquely targeting SLC1A5. We therefore hypothesized that protein deglycosylation could be one of the cellular mechanisms triggered by the pathogen to favor its intracellular adaptation and multiplication. We thus looked whether *F. tularensis* LVS infection had a general impact on known glycosylation pathways. We first demonstrated, using a specific microarray, that *F. tularensis* LVS induced the modification of numerous eukaryotic genes involved in both N- and O-glycosylation pathways. Remarkably, we found that, as soon as 1 h after *F. tularensis* LVS infection, there was a dramatic increase of O-glycosylation, mainly on proteins in the 70 kDa range. This glycosylation decreased after 5 hours infection. We then used mass spectrometry to characterize the 70 kDa O-glycosylated proteins. One of the proteins identified was the heat-shock protein BIP (also called HspA5 or Grp78). We found that *F. tularensis* LVS infection up regulated BIP both at transcriptional and post-translational levels. Remarkably, BIP expression was also required to support *F. tularensis* LVS intracellular multiplication. BIP is a multifunctional ER chaperone involved in the regulation of multiple cellular pathways. We found that BIP up-regulation by *F. tularensis* LVS modulated both the unfolded protein response (UPR) and the Hexosamine biosynthetic pathway. Altogether, our data suggest that the effects exerted by *F. tularensis* on the UPR may participate in the dampening of autophagy in human cells.

S7-4

Complement C3 as a determinant of macrophage death during *Francisella* infection

Susan Brock and Michael Parmely

Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS, USA

Macrophage death is a defining feature of *Francisella tularensis* infection both in vitro and in vivo. However, the significance of this outcome is not completely understood. Opsonization with complement and the complement receptor CR3 are important mediators of optimal *Francisella* uptake by macrophages. Because the signals leading to cell death of infected macrophages are still unknown, we asked whether complement deposition on *F. tularensis* strain Schu S4 is important for initiating macrophage death following infection. As previously reported by others, we found that opsonization with fresh human serum led to covalent deposition of iC3b on the surface of Schu S4, which did not occur in the presence of heat-inactivated serum (ΔHS, 56°C for 30 minutes) or C3-depleted human serum. Fresh serum enhanced uptake into primary human macrophages and led to high intracellular bacterial burdens and host cell death. Neither ΔHS nor C3-depleted serum enhanced uptake or induced macrophage death. Serum opsonization of the phagosomal escape-deficient mutants Schu S4 ΔfevR and Schu S4 ΔiglC led to wild-type iC3b deposition and enhanced bacterial uptake, but failed to induce macrophage death. These findings suggest that *Francisella* uptake via complement receptors is not sufficient to induce host cell death, whereas bacterial escape into the cytosol is required. Complementation of Schu S4 ΔfevR with a fevR plasmid restored intracellular replication and the ability of the mutant to induce host cell death. Serum opsonization of strain Schu S4 ΔpurMCD, a mutant that is able to escape the phagosome but shows limited cytosolic replication, also led to macrophage death. This observation suggests that bacterial replication in the cytosol is not required for the induction of death in *F. tularensis* infected cells, and implies that cell death signaling occurs fairly early following uptake and phagosomal escape. We are currently investigating three potential mechanisms by which C3 might promote macrophage death: (1) C3 promotion of phagosome disruption, (2) C3 facilitation of initial high cytosolic bacterial burdens, or (3) engagement of C3 that is bound to the bacterial surface by a host cytosolic C3 sensor. Regardless of the precise mechanism, our findings suggest that human macrophage death induction by *F. tularensis* requires the presence of C3-coated bacteria in the cytosol, implying that complement plays a significant role in regulating macrophage fate during *F. tularensis* infections.

S7-5

Intestinal goblet cells can augment M-cells for entry of oral vaccines

Aimee L. Cunningham[1], M. Neal Guentzel[1], Jieh-Juen Yu[1], Karl E. Klose[1], Tonyia D. Eaves-Pyles[2], and Bernard P. Arulanandam[1]

[1] South Texas Center for Emerging Infectious Disease and the Center for Excellence in Infection Genomics, University of Texas at San Antonio, San Antonio, TX; [2] Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA

M-cells (microfold cells) are thought to be a primary conduit of intestinal antigen trafficking. However, transient depletion of M-cells in mice with anti-RANKL (Receptor Activator of NF-κB Ligand) at the time of oral *Francisella novicida* ΔiglB immunization did not significantly reduce protective efficacy against lethal *F. novicida* U112 and LVS intranasal challenge suggesting complementary mechanism(s) may be involved in vaccine entry through the intestinal mucosa. Thus, we examined other possible gastrointestinal antigen sampling mechanisms and found that ΔiglB co-localized to goblet cells (GC) by confocal microscopy 90 minutes after oral administration. Further studies using human HT29 cells, which can be transformed to a GC phenotype demonstrated that U112 and ΔiglB were rapidly internalized, with concomitant evidence of significant intracellular replication of U112, but not ΔiglB. Goblet-like cells and associated exosomes pulsed with ΔiglB induced activation of PBMC-derived dendritic cells (DC) leading to the production of IL-1β and IL-8 suggesting that both direct and indirect interactions of GCs with DCs may lead to DC activation following uptake of particulate antigens (i.e., △iglB) by GCs. Collectively, this study provides new insight into the role of goblet cells in intestinal antigen trafficking and additional complementary pathways that may be involved in the generation of optimal immunity following oral vaccination.

Session 7:

Host responses to *Francisella* Part B

Session 7: Host responses to *Francisella* Part B

Chairs: Siobhán Cowley
CBER/FDA, USA

Bradley Jones
University of Iowa, USA

S7-6 Correlates of protection against *Francisella tularensis*: are we there yet?
Roberto De Pascalis

S7-7 *Francisella tularensis* virulence: still scratching at the surface
Wayne Conlan and Anders Sjostedt

S7-8 Evaluation of pathogenesis and immune response of *Francisella tularensis* in cottontail rabbits
Vienna R. Brown, Helle Bielefeldt-Ohmann, Francisco Olea-Popelka, Paul W. Gordy and Richard A. Bowen

S7-9 Characterization of the live attenuated Schu S4Δ*aroD* vaccine candidate against pulmonary tularemia
Aimee L. Cunningham, Araceli E. Santiago, Barbara J. Mann, Aiping Qin, Christen Grassel, Stefanie N. Vogel and Eileen M. Barry

S7-10 Dissection of *Francisella* virulence in *Dictyostelium;* a genetically tractable host system
Elisabeth O. Lampe, Lydia Herrmann, Yannick Brenz, Carl Zingmark, Anders Sjöstedt, Hanne C. Winther-Larsen and Monica Hagedorn

S7-6

Correlates of protection against *Francisella tularensis*: Are we there yet?

Roberto De Pascalis

Laboratory of Mucosal Pathogens and Cellular Immunology, DBPAP, CBER, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring 20832, Maryland, U.S.A.

Tularemia is not frequent in nature, and novel vaccines against *F. tularensis* most likely cannot be assessed by large-scale clinical trials. Therefore, the identification of correlates of protection would be useful to monitor immune responses after vaccination against *F. tularensis*. Measuring T-cell immune responses, which are predominantly induced by *F. tularensis,* is more challenging than measuring antibody immune responses, and many different methods have been used to quantify T-cell immune-related factors. To derive useful correlates of protection against *F. tularensis*, our approach focuses on identifying multiple immune factors that, in a multivariate analysis, can overcome biologic and technical variabilities. Our method allowed us to establish similarities and differences between tissue samples, vaccination routes, and mouse strains, and thereby identify potential correlates of protection. In addition, to validate our approach, we extended our studies to a different animal species of current interest, namely Fisher rats. Rats better discriminate between Type A and Type B *F. tularensis* infection than mice, and develop pathology similar to humans. Ongoing studies are evaluating the performance of this approach by screening new vaccine candidates, with the goal of ultimately defining a robust panel of correlates of protection. Ideally, this panel may include T cell-related functions combined with measurements of specific anti-*F. tularensis* antibodies, to predict the outcome of vaccination and to bridge between animal models and humans.

S7-7

Francisella tularensis virulence: still scratching at the surface

Wayne Conlan[1] and Anders Sjostedt[2]

[1] National Research Council of Canada, Human Health Therapeutics Portfolio, 100 Sussex Drive, Ottawa, Ontario K1A 0R6, Canada; [2] Umea University, Chief, Dept. of Clinical Microbiology and Bacteriology, Umea, Sweden 90185.
e-mail: anders.sjostedt@climi.umu.se

The 21st century has truly been a golden age for tularemia research. Hundreds of scientists and hundreds of millions of dollars have been devoted to unravelling the multitude of secrets of *Francisella tularensis* infection and immunity, ecology, genetics and physiology leading to expectations of better vaccines, therapies, diagnostics, detection techniques, etc. Whilst many of these practical spin-offs are emerging, our overall understanding of *F. tularensis* virulence remains relatively superficial. This is more a reflection of the complexity of the bacterium rather than failure by the scientific community. Our understanding of the mechanisms of cell invasion, the *Francisella* pathogenicity island, and host immunosuppression has made impressive strides. Nevertheless, there is much we don't know about how *F. tularensis* interacts with the host to cause disease. I will scratch the surface of our ignorance using personal examples of mysteries revealed, but not pursued. Among other things, this will include the identity of skin and splenic host cells that harbour the pathogen, and the various unusual *in vivo* behaviours of some of the SCHU S4 deletion mutants we have generated over the years, but set aside in pursuit of a live attenuated vaccine. I hope to demonstrate that our field will remain fertile ground for discovering important new insights about *F. tularensis* virulence for many years to come, subject to funding.

S7-8

Evaluation of pathogenesis and immune response of *Francisella tularensis* in cottontail rabbits

Vienna R. Brown, Helle Bielefeldt-Ohmann, Francisco Olea-Popelka, Paul W. Gordy, Richard A. Bowen

Colorado State University, Colorado State University-163 Campus Delivery, Fort Colins, CO, United States

Francisella tularensis is a highly virulent, zoonotic bacterium that causes significant natural disease and is also of concern as an organism for bioterrorism. Serologic testing of wildlife is frequently used to monitor spatial patterns of infection and quantify exposure. Cottontail rabbits are a natural reservoir for this bacterium in the U.S., although very little work has been done experimentally to determine how these animals respond to infection; thus, information gathered from field samples can be difficult to interpret. The objective of this study was to provide an initial characterization of clinical disease, bacteremia, pathology, and antibody kinetics of Desert cottontail rabbits (*Sylvilagus audubonii*) experimentally infected with five strains of *F. tularensis*. We infected rabbits with four field strains, including MA00-2987 (A1b strain), WY96-3418 (type A2), KY99-3387 and OR96-0246 (type B strains), and with SchuS4 (type A1a strain), a widely used, virulent laboratory strain. The results clearly indicate that infection with different strains of the bacterium resulted in significantly varied patterns of disease as well as gross and histopathology. We also characterized long term humoral immune responses and the ability of cottontail rabbits to clear infection of type B strains of *F. tularensis.* Furthermore, we found that previous infection with a type B strain afforded partial protection against challenge with a virulent type A strain. Understanding *F. tularensis* infection in a natural reservoir species can guide sero-surveillance projects as well as generate new insights into environmental maintenance of this pathogen.

S7-9

Characterization of the live attenuated SCHU S4Δ*aroD* vaccine candidate against pulmonary tularemia

Aimee L. Cunningham[1], Araceli E. Santiago[2], Barbara J. Mann[3], Aiping Qin[3], Christen Grassel[1], Stefanie N. Vogel[4] and Eileen M. Barry[1]

[1] Center for Vaccine Development, University of Maryland Baltimore, Baltimore, MD; [2] Department of Pediatrics and [3] Department of Medicine, University of Virginia, Charlottesville, VA; [4] Department of Microbiology and Immunology, University of Maryland Baltimore, Baltimore, MD 685 West Baltimore Street, Baltimore, MD 21201, USA

Our goal is to develop a live attenuated tularemia vaccine generated from the highly human virulent subspecies *tularensis* that induces protective immunity against subsequent pulmonary challenge. We have created and characterized a vaccine candidate, Schu S4Δ*aroD*, that has shown promise by generating protection against WT Schu S4 in both mice and rabbit models. Schu S4Δ*aroD* lacks the 3rd enzyme of the shikimate pathway for aromatic amino acid synthesis and is attenuated *in vitro* for growth in broth culture, in murine J774 and primary peritoneal macrophages, and *in vivo* (intranasal LD_{50} in C57BL/6 mice ≈ $5x10^5$ CFU). In murine peritoneal and human THP-1 macrophages, Schu S4Δ*aroD* is deficient for replication and is rapidly cleared. Despite this deficit, Schu S4Δ*aroD* infection induced comparable acute cytokine responses to those generated by WT Schu S4; TLR2, TNF-α, IL-1β, KC, iNOS, and RANTES were induced at equivalent levels in murine macrophages and TNF-α, IL-12, TGF-β, IL-1β, and IL-18 were similarly induced in THP-1 macrophages. Further evaluation in mice determined that Schu S4Δ*aroD* colonizes organs in a dose-dependent manner; peak bacterial burdens in lungs and liver were reached on days 3 or 7 based on initial inoculum, with clearance by 21 days. Interestingly, dose-dependent histopathology (again based on initial inoculum) was observed within the liver on day 7 when all animals had identical bacterial organ loads. The kinetics of cytokine induction in organs correlates directly with bacterial burdens, as reaching peak burden corresponds to spikes in cytokine and chemokine production in lungs and liver (TNF-α, Cox-2, IL-1β, KC, MCP-1, iNOS, IL-10, IL-17, IFN-γ, and T-bet). We hypothesize that an early (day 3) inflammatory response in mice is detrimental, as cytokine induction patterns mimic that of WT (greater inflammation is seen and morbidity and mortality are increased). In contrast, a later response (day 7) shows decreased pathology, increased clearance, and protection against subsequent challenge. Importantly, mice receiving higher booster vaccination doses (10^6 CFU, instead of 10^5 CFU or no boost) completely cleared WT *F. tularensis* (Schu S4 or KY99-NR647) and did not show signs of splenomegaly. Overall, these studies suggest that Schu S4Δ*aroD* serves as a promising vaccine candidate, generating protection against 2 wild-type *Ft* strains in mice. Ongoing studies will characterize cellular and humoral responses in both animal models, and will seek to determine which arm of the immune system best helps to control intracellular replication of WT Schu S4.

S7-10

Dissection of *Francisella* virulence in *Dictyostelium;* a genetically tractable host system

Elisabeth O. Lampe[1,2], Lydia Herrmann[3], Yannick Brenz[3], Carl Zingmark[4], Anders Sjöstedt[4], Hanne C. Winther-Larsen[1,2] and Monica Hagedorn[3]

[1]Center of Integrative Microbial Evolution (CIME); [2]Department of Pharmaceutical Biosciences, University of Oslo, PO Box 1068 Blindern, 0316 Oslo, Norway; [3]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany and [4]Department of Clinical Microbiology, Umeå University, Umeå, Sweden

Francisella is one of the most infectious bacterial agents known where less than 10 CFU can cause severe invasive disease in both vertebrates and invertebrates. The genus is broadly divided into two lineages; *F. tularensis* subsp that cause tularemia and *F. noatunensis* subsp that cause francisellosis in aquatic organisms. Upon infection Francisella invades and replicate in a number of cell types where macrophages are an important target. *Francisella* has also been identified in protists. The professional phagocyte *Dictyostelium discodeum* could therefore be an attractive model system to dissect host-pathogen interactions. Here the establishment of a *Dictyostelium–Francisella* infection model is described using the fish pathogen *F. noatunensis* subsp *noatunensis* (*F.n.n*) which has the same optimal growth temperature as *Dictyostelium*. Within mammalian macrophages *Francisella* will after cell entry escape from the *Francisella*-containing phagosomes into the host cell cytosol where it replicates thereby avoiding phagolysosomal fusion and degradation. Mutations in the *iglC* gene, located within the *Francisella* pathogenicity island, impair *Francisella* in phagosomal escape and intracellular growth. Within *Dictyostelium,* wild-type *F.n.n* is initially associated with the endosome marker p80 and vATPase, a proton pump for phagosomal acidification. This colocalization is disrupted at 6 hrs post infection illustrating that *F.n.n* is able to escape from phagosomal maturation and into the cytosol where it is able to persist. This behavior is in contrast to the *ΔiglC* mutant which after entry most bacteria is efficiently exocytosed within 2 hrs post infection. The mutants that are still present within *Dictyostelium* fail to translocate into the host cell cytosol and follows the normal phagosomal maturation pathway. Taken together these results mimicking the situation reported for *Francisella* in other systems and illustrate the power of the *Francisella-Dictyostelium* infection model.

Session 8:

Vaccines, Therapeutics and Diagnostics for Tularemia

Session 8: Vaccines, Therapeutics and Diagnostics for Tularemia

Chairs: Anders Sjöstedt
University of Umeå, Sweden

Wayne Conlan
National Research Council, Canada

S8-1 Development of efficacious Schu S4-based live attenuated *Francisella* vaccine strains
Eileen M. Barry, Barbara J. Mann, Araceli Santiago, Douglas S. Reed and Aimee Cunningham

S8-2 The role of B cells in the course of *F. tularensis* infection
Zuzana Krocova

S8-3 Antibiotic resistance in *Francisella tularensis*: *in vitro* and *in vivo* evaluation
Max Maurin, Yvan Caspar, Vivien Sutera, Sandrine Boisset, Gautier Hoarau, Brieuc Gestin, Patricia Renesto and Dominique Schneider

S8-4 Development of a novel vaccine against tularemia
Ping Chu, Aimee L. Cunningham, Jesse Nguyen, Jieh-Juen Yu, Jeffrey R. Barker, C. Rick Lyons, Julie Wilder, Michelle Valderas, Robert L. Sherwood, Bernard P. Arulanandam and Karl E. Klose

S8-5 Protection with a glyco-conjugate vaccine against aerosol delivered *Francisella tularensis* Schu S4 in Fischer 344 rats
L.E. Marshall, A.O. Whelan, M.N. Nelson, C. Denman, M.G. Moule, T.P. Atkins, B.W. Wren and J.L. Prior

S8-1

Development of efficacious SCHU S4-based live attenuated *Francisella* vaccine strains

Eileen M. Barry[1], Barbara J. Mann[2], Araceli Santiago[3], Douglas S. Reed[4] and Aimee Cunningham[1]

[1]Center for Vaccine Development, University of Maryland Baltimore, Baltimore, MD; [2]Department of Pediatrics and [3]Department of Medicine, University of Virginia, Charlottesville, VA;[4]; and [5]Department of Infectious Diseases and Immunology, University of Pittsburgh, Pittsburgh, PA685 W. Baltimore St., Baltimore, MD 21201, USA

There is no licensed vaccine to prevent disease caused by *F. tularensis*, a category A select agent that can cause infection with an aerosol dose as low as 10 CFU. Volunteer studies reported in the 1960's demonstrated that the Live Vaccine Strain (LVS), an attenuated type B strain, provided partial protection against aerosol challenge with the highly virulent type A strain, providing proof of principle that a live attenuated strain may induce protection against type A organisms, but that improved efficacy is required. We hypothesized that a live vaccine based on a type A strain would provide a higher level of protection. A series of ten new live vaccine candidates, based on type A strain Schu S4, that contain precisely defined deletions in genes encoding virulence factors or metabolic enzymes were constructed. Six of the mutant strains, containing deletions in genes encoding guanine nucleotide or aromatic amino acid biosynthesis enzymes, were highly attenuated for virulence in mouse and rabbit models. These attenuated strains also demonstrated deficits in macrophage survival and replication. Evaluation in the mouse model demonstrated that Schu S4Δ*gua* strains were not protective against type A challenge. This is in contrast to LVSΔ*gua* mutant strains, which fully protected against lethal LVS challenge. One candidate strain, Schu S4Δ*aroD*, was protective against a high challenge dose of wild type (WT) type A strain Schu S4 in the mouse model. We further showed that Schu S4Δ*aroD* colonizes the lungs and livers of mice following intranasal immunization in a dose-dependent manner and that the subsequent cytokine profile suggests a kinetic pattern that is associated with safety and protection. Further studies utilizing our recently established rabbit model confirmed the attenuation of both Schu S4Δ*guaAB* and Schu S4Δ*aroD*. In this model, immunization with Schu S4Δ*guaAB* conferred 27% protection and significantly increased time to death following aerosol challenge with Schu S4. Immunization with Schu S4Δ*aroD* conferred 50–86% protection against challenge depending on the vaccine regimen with aerosol prime-boost providing the highest level of efficacy. These data suggest that Schu S4Δ*aroD* is a promising vaccine candidate for further evaluation. Furthermore, our series of vaccine strains that are attenuated but confer a spectrum of protective efficacy serves as a valuable tool for the identification of immune responses associated with protection. These studies underscore differences between vaccine candidates derived from different parent strains and the value of evaluation using different animal models.

S8-2

The role of B cells in the course of *F. tularensis* infection

Zuzana Krocova

FMHS, UoD, Hradec Kralove, Czech Republic

A general characteristic of bacterial intracellular pathogens, such as *Brucellae*, *Listeriae*, *Salmonellae*, and *Francisellae*, is adhesion to, internalization into, and proliferation within professional phagocytes. Macrophages are considered to be the preferred target. In addition to infecting professional phagocytes, however, intracellular bacteria also attack non-phagocytic cells, and also into B cells. Internalization alone, from the side of the host cell, can be mediated by different cell surface receptors depending upon the conditions under which the process is occurring. Actin rearrangement and active microtubules finalize the internalization process. The bind of bacteria to B cell receptor, the complement receptors CR1/2, CR3, and CR4 as well as Fc receptors leads to the substantial modulation of intracellular trafficking and final fate of bacteria inside the phagocyte, B cell including. Recently, we documented that *Francisella* infect *in vitro* murine and human B cell lines and *in vivo* murine peritoneal as well as spleen B cells. Here, we demonstrate that *Francisella tularensis* subsp. *holarctica* strain LVS (FSC155) significantly infects subtypes of murine peritoneal B cells early after intraperitoneal infection. The uptake of *Francisella* into B cells is mediated by the BCR and CRs with active participation of *Francisella* itself. Using the murine model of tularemia we presented here that *F. tularensis* LVS infects early after intra-peritoneal *in vivo* infection $CD19^+$ cells, exclusively B-1a cells and activate them to express activation markers MHC class II, CD25, CD54, CD69, and co-stimulatory molecules CD80 and CD86. As early as 12 hours post infection the peritoneal $CD19^+$ cells produce IFN-γ, IL-1β, IL-4, IL-6, IL-12, IL-17, IL-23, and TNF-α. The spleen $CD19^+$ cells respond to infection with some delay. Moreover, infected A20 B cell line presents *F. tularensis* antigens to spleen $CD3^+$ cells. Thus, the data presented here entitle us to believe that B cells have all the attributes to actively participate on the induction and regulation of adaptive immune response in the course of early stages of *F. tularensis* infection.

S8-3

Antibiotic resistance in *Francisella tularensis*: *in vitro* and *in vivo* evaluation

Max Maurin[1,2], Yvan Caspar[1,2], Vivien Sutera[1], Sandrine Boisset[1,2], Gautier Hoarau[1], Brieuc Gestin[1], Patricia Renesto[2] and Dominique Schneider[2]

[1] Centre National de Référence des Francisella, Institut de Biologie et de Pathologie, Département des Agents Infectieux, Centre Hospitalier Universitaire de Grenoble, France; [2] LAPM, CNRS/UJF UMR 5163, Université Grenoble Alpes, Grenoble, France

Francisella tularensis, a gram-negative, facultative intracellular bacterium, is the etiological agent of tularemia. This zoonosis may manifest by regional lymphadenopathies *(i.e.*, the ulceroglandular, glandular, oculoglandular and oropharyngeal forms), or systemic diseases (the pneumonic and typhoidal forms). *F. tularensis* is a highly infectious pathogen, and a potential bioterrorism agent (CDC class A). It is naturally resistant to many antibiotic compounds. Only three antibiotic classes are currently recommended for treatment of tularemia: the aminoglycosides (especially streptomycin and gentamicin), the tetracyclines (*e.g.*, doxycycline), and the fluoroquinolones (*e.g.*, ciprofloxacin). The aminoglycosides are used only for severe systemic diseases, while the fluoroquinolones are advocated as first-line treatment for common tularemia cases and post-exposure prophylaxis of tularemia. Because all three antibiotic classes may have severe side effects in young children and pregnant women, the macrolides (especially azithromycin) have been occasionally used as an alternative for diseases of mild to moderate severity, and in geographic areas were the naturally macrolide-resistant biovar 2 strains of *F. tularensis* subsp. *holarctica* are absent. No acquired resistance to these four antibiotic classes have been reported so far in natural strains of *F. tularensis* isolated from animals, humans, arthropods or various environments. However, treatment failures and relapses are commonly reported in tularemia patients, especially those suffering from lymph node suppuration. This might suggest *in vivo* selection of antibiotic resistance in the corresponding patients. Moreover, intentional release of antibiotic-resistant strains of *F. tularensis* represents a serious threat for human and animal health. Using *Francisella* strains with attenuated virulence for humans, we investigated the potential for *in vitro* selection of antibiotic resistance and multi-drug resistance (MDR) in this group of microorganisms. We selected multiple lineages of resistant mutants, and fully characterized both their resistance phenotypes and the involved genetic mechanisms. Using a macrolide or a fluoroquinolone as the selective antibiotic compound, we easily obtained not only high-level resistant mutants, but also mutants with cross-resistance to tetracyclines and aminoglycosides. Although we found various combinations of mutations in the genes encoding known antibiotic targets, overexpression of efflux pumps also occurred. As for fluoroquinolones, we developed molecular tools for detection

of antibiotic-resistance mutations in *F. tularensis* strains isolated from tularemia patients, but also directly in clinical samples collected in infected patients before and after fluoroquinolone therapy. We could not detect the previously characterized major fluoroquinolone-resistance mutations in the investigated strains and for a limited number of clinical samples collected in patients suffering from failures or relapses after fluoroquinolone therapy. Our strategy to assess the potential of *in vivo* selection of antibiotic resistance in *F. tularensis* should be further evaluated using a larger collection of clinical samples, and extended to other antibiotic compounds and/or resistance mechanisms. Moreover, the developed molecular tools might now be used in the context of bioterrorism for rapid detection of antibiotic resistant strains.

S8-4

Development of a novel vaccine against tularemia

Ping Chu[1], Aimee L. Cunningham[1], Jesse Nguyen[1], Jieh-Juen Yu[1], Jeffrey R. Barker[1], C. Rick Lyons[2], Julie Wilder[3], Michelle Valderas[3], Robert L. Sherwood[3], Bernard P. Arulanandam[1] and Karl E. Klose[1*]

[1] South Texas Center for Emerging Infectious Diseases and Dept. of Biology, University of Texas San Antonio, San Antonio TX; [2] Colorado State University, Fort Collins CO; [3]Lovelace Respiratory Research Institute, Albuquerque, NM, USA

Francisella tularensis causes the disease tularemia. Human pulmonary exposure to the most virulent form, *F. tularensis* subsp. *tularensis* (Ftt), leads to high morbidity and mortality, resulting in this bacterium being classified as a potential biothreat agent. However, a closely-related species, *F. novicida*, is avirulent in healthy humans. No tularemia vaccine is currently approved for human use. We demonstrate that a single dose vaccine of a live attenuated *F. novicida* strain (Fn *iglD*) protects against subsequent pulmonary challenge with Ftt using two different animal models, Fischer 344 rats and cynomolgus macaques (NHP). The Fn *iglD* vaccine showed protective efficacy in rats, as did a Ftt *iglD* vaccine, suggesting no disadvantage to utilizing the low human virulent *Francisella* species to induce protective immunity. Comparison of specific antibody profiles in vaccinated rat and NHP sera by proteome array identified a core set of immunodominant antigens in vaccinated animals. This is the first report of a defined live attenuated vaccine that demonstrates efficacy against pulmonary tularemia in a NHP, and indicates that the low human virulence *F. novicida* functions as an effective tularemia vaccine platform.

S8-5

Protection with a Glyco-conjugate vaccine against aerosol delivered *Francisella tularensis* SCHU S4 in Fischer 344 rats

L.E. Marshall[1], A.O. Whelan[1], M.N. Nelson[1], C. Denman[2], M.G. Moule[2], T.P. Atkins[1,3], B.W. Wren[2] and J.L. Prior[1,3]

[1] Defence Science and Technology Laboratory, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK; [2] Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine; Keppel Street, London WC1E 7HT, UK; [3] School of Biosciences, University of Exeter, Devon, UK

There is a requirement for an efficacious, licensable vaccine to protect against tularemia. The lipopolysaccharide (LPS) of *Francisella tularensis* is sub-optimally protective against a parenteral lethal challenge in mice. We have previously shown that conjugation of LPS to *Pseudomonas aeruginosa* exoprotein A, using a novel bio-synthetic technique, improves protection of the resulting vaccine candidate in mice, compared to LPS alone. Recent studies have shown that a pulmonary model of tularemia in Fischer 344 rats may be a more relevant animal model in which to test vaccines to protect against this disease. Therefore, a Fischer 344 rat model of aerosol delivered *F. tularemia* Schu S4 in which to test the glyco-conjugate vaccine candidate has been developed. In order to characterise a protective immune response in these rats, Live Vaccine Strain was delivered by the sub-cutaneous route, and was shown to be fully protective against a *F. tularensis* Schu S4 aerosol challenge. LVS was shown to invoke a robust memory immune response within 21 days of vaccination, extended to 35 days post vaccination. In order to test efficacy of the glyco-conjugate in this model, three doses were administered to rats at two weekly intervals, followed by challenge with *F. tularensis* Schu S4 by the aerosol route five weeks post final vaccination. All vaccinated animals survived challenge, and showed no signs of disease, whilst the majority of non-vaccinated control animals succumbed to infection, and all showed severe signs of disease. Vaccinated animals lost no weight and did not develop a fever, compared to up to 15% weight loss and fever in all non-vaccinated control animals. The cell mediated immune response to the glyco-conjugate in rats was also characterised.
This is the first demonstration of protection against pulmonary tularemia in rats using a sub-unit vaccine.

Session 9:

Poster session C

Session 9: Poster session C

S9-1 Recombinant attenuated *Francisella tularensis* live vaccine strain *capb* mutant overexpressing type vi secretion system proteins induces improved protection against pneumonic tularemia in mice
Qingmei Jia, Richard Bowen, Barbara Jane Dillon and Marcus A. Horwitz

S9-2 Seroprevalence of tularemia among healthy individuals in endemic regions of Georgia
Nino Akhvlediani, Irma Burjanadze, Marina Topuridze, Archil Navdarashvili, Ciuri Tushishvili, David Baliashvili, Sopio Dolbadze, Mariam broladze, Nazi Chitadze, Tamar Chikviladze, Paata Imnadze, Tinatin Kuchuloria, Tamar Akhvlediani, Louise-Anne McNutt, Robert Rivard, and Gvantsa Chanturia

S9-3 Extremely successful re-evaluation of Pavlovich's broth medium T for growth of *Francisella tularensis* ssp.
Silke Becker, Petra Lochau, Daniela Jacob, Klaus Heuner, Roland Grunow

S9-4 Results on antimicrobial susceptibility testing of *Francisella tularensis* using minimal inhibitory concentration with two methods, gradient diffusion and broth dilution
Daniela Jacob, Massimo Fabbi, Paola Prati, Silke Becker, Petra Lochau, Roland Grunow

S9-5 Meropenem resistance in the MRIGlobal *Francisella tularensis* repository
Robyn Kelly, Sara Hamilton, Deandra Audley, Aaron Tyler, H. Carl Gelhaus

S9-6 Experimental basis of rational treatment of tularemia
N.V. Pavlovich, M.V. Tsimbalistova

S9-7 Entry of *Francisella tularensis* into B cells
Lenka Plzakova, Klara Kubelkova, Zuzana Krocova, Ales Macela

S9-8 Characterisation and exploitation of RelA as a novel antibacterial target for the biowarfare agent *Francisella tularensis*
Rachael C. Wilkinson, Petra C.F. Oyston, and Peter L. Roach

S9-9 Immunodiagnostic and molecular methods in a twelve-year follow-up of patients suffering from tularemia in Serbia
Marina Djordjevic-Spasic, Herbert Tomaso

S9-10 Effect of Fe-superoxide dismutase activity on the biological properties of the *Francisella tularensis* 15 vaccine strain
Sukhova M.A., Kravchenko T.B., Mokrievich A.N., Pavlov V.M.

S9-11 Analysis of *Francisella*-immune murine peripheral blood leukocytes reveals new insights in the identification of potential correlates of protection against *Francisella tularensis*
Lara Mittereder, Roberto De Pascalis, Nikki J. Kennett and Karen L. Elkins

S9-12 Data mining and genetic studies of *Francisella tularensis* in search of novel tularemia vaccine candidates
Galia Zaide, Naomi Ariel, Itzhak Inbar, Uri Elia, Hila Cohen, Gila Friedman, Anat Zvi, Avigdor Shafferman and Ofer Cohen

S9-13 Synthetic bis-indolic derivatives with antibacterial activity against *Francisella tularensis* subspecies *holarctica*
Yvan Caspar, Jean-Noël Denis, Frédéric Minassian and Max Maurin

S9-14 *Vaccine-mediated protection against Francisella tularensis*
Igor Golovliov, Kjell Eneslätt, Helena Lindgren and Anders Sjöstedt

S9-15 Limited potential of highly virulent *F. tularensis* strain to induce protective immunity against *F. tularensis* challenge
Erez Bar-Haim

S9-16 A poly-epitope DNA vaccine engineered on the basis of *Francisella tularensis* whole-genome immunoinformatic analysis elicits a protective CTL immune response
S. Rotem

S9-17 Molecular evidences of *Francisella tularensis* in rodents in Iran
Abdolrazagh Hashemi Shahraki, Ehsan Mostafavi, Ahmad Ghasemi, Ahmad Mohamoudi, Jamshid Darvish, Mansour Aliabadian

S9-18 Serological survey of tularemia in rodents in Western Iran
E. Mostafavi, A. Hashemi Shahraki, AR. Japoni-Nejad, S. Esmaeili, MM. Gouya, J. Darvish, MM. Sedaghat, A. Mohammadi, ZA. Mohammadi, A. Mahmoudi, B. Pourhossein, M. Gyuranecz

S9-19 Whole-genome sequencing of Italian isolates of *Francisella tularensis* subsp. *holarctica*: preliminary data*Nadia Vicari, Erika Scaltriti, Chiara Chiapponi, Carmen Mandalari, Andrea Manfredini, Stefano Pongolini, Massimo Fabbi*

S9-20 Study of the biological threats for *F. tularensis* distribution in environmental objects
Stegniy B.T., Gerilovych A.P., Zavgorodniy A.I., Obykhovska O.V., Arefiev V.L.

S9-21 The influence of metal ions on the growth of *Francisella tularensis subsp. novicida* in water
Tea Jelacic, Andrea Majstorovic, Martina Dajak, Mateja Ozanic, Valentina Marecic and Marina Santic

S9-22 Spatial distribution of *Francisella tularensis* in selected districts of Punjab province, Pakistan
Javed Muhammad, Masood Rabbani, Muhammad Zubair Shabbir, Khushi Muhammad, Muhammad Wasim, Malik Zia Ul Hassnain, Haroon Rasheed Chaudhry, Muhammad Tasleem Ghori, Muhammad Haisem, Muhammad Asad Ali, Tariq Jamil, Girish S Kirimanjeswara, Bhushan M Jayarao

S9-23 Protracted course of human tularemia
Roland Grunow, Insa Joost, Annerose Serr, Cansel Yildiz, Philipp Kurz, Silke Klee, Klaus Heuner, Daniela Jacob

S9-24 Improved real-time PCR detection of *Francisella tularensis* in challenging waters
Elin Nilsson, Linda Jansson, Anna Macellaro, Peter Rådström, Johanna Thelaus, Johannes Hedman and Laila Noppa

S9-25 First case of *Francisella tularensis* subsp. mediasiatica isolation in Altai, south part of Siberia
A.N.Mokrievich, V.S.Timofeev, T.Yu.Kudryavtseva, G.I.Ulanova, S.B.Karbysheva, R.I.Mironova, G.M.Vakhrameeva, T.I.Gubareva, V.M.Pavlov, I.A.Dyatlov

S9-26 Modeling of tularemia cases within major endemic regions of Sweden 1984–2012
A. Desvars , X. Liu , A. Sjöstedt , A. Johansson , P. Rydén

S9-27 Reemergence of tularemia in the Netherlands
Miriam Koene, Joke vd Giessen, Jolianne Rijks, Miriam Maas, Daan Notermans, Ewout Fanoy, Marc Engelsma, Arjan Stroo, Peter van Tulden, Kitty Maassen, Corien Swaan, Mauro De Rosa, Ton Oomen, Frans Reubsaet, Andrea Gröne, Hendrik Jan Roest

S9-28 Epidemic tularemia outbreak in Russia (Khanty-Mansi autonomous okrug) in 2013
A.V. Mazepa, A.K. Noskov, A.V. Kholin , S.V. Balakhonov, E.S. Kulikalova, I.I. Kozlova

S9-29 Genome based analysis of the Swiss *Francisella tularensis holarctica* population
Matthias Wittwer, Christian Beuret, Nadia Schürch

S9-30 Presence of *Francisella tularensis* in mosquitoes of central Poland
K. Formińska, A. Masny, E.Gołąb, A.Zasada

S9-31 *Yersinia pestis* and *Francisella tularensis* in vectors and rodents in Northern Azerbaijan
Huseyn Hajiyev, Bakhtiyar Aydinov, Azad Jafarov

S9-32 *Francisella* species in ticks and animals, Iberian Peninsula
Lopes de Carvalho I, Toledo A, Carvalho CL, Barandika JF, Respicio-Kingry LB, Garcia-Amil C, García-Pérez AL, Olmeda AS, Zé-Zé L, Petersen JM, Anda P, Núncio MS, Escudero R

S9-33 A glandular tularemia case report from an unusual geographic location in Georgia
Tinatin Kuchuloria, Marina Endeladze, Nazibrola Chitadze, Tamar Chikviladze, Mariam Broladze, Natalia Abazashvili, Tamar Giorgadze, Paata Imnadze, Tengiz Tsertsvadze, Nino Akhvlediani, Tamar Akhvlediani, Louise-Anne McNutt, Robert G. Rivard, Gvantsa Chanturia

S9-34 Field planning based on ecological niche models of *Francisella tularensis* isolated from *Microtus arvalis* and *Dermacentor marginatus* in Georgia
I. Burjanadze, N. Tsertsvadze, M. Shavishvili, I. Shalutashvili, A. Dzneladze, A. Navdarashvili, G. Chanturia, P. Imnadze, I. Kracalik, J. K Blackburn

S9-35 Tularemia in children in Kosovo from November 2014-March 2015
Sadiku I.,Bajrami M., Ramadani H., Mehmeti M., Ponosheci A., Berisha V.

S9-36 *Francisella tularensis* clades B.FTN002-00 and B.13 are associated with distinct pathology in the European brown hare (*Lepus europaeus*)
Francesco C. Origgi, Paola Pilo

S9-37 Microbial genomic analysis indicate environmental persistence of tularemia in Bulgaria over three decades of epidemiological silence
Kerstin Myrtennäs, Krustyu Marinov, Anders Johansson, Edvin Karlsson, Mona Byström, Mats Forsman

S9-38 Performance of serological tests for diagnosis tularemia in Peja region, January- April 2015
Vanina Kryeziu-Gafuri, Fatmire Kollçaku, Shqipe Krasniqi-Kola

S9-39 Tularaemia in Peja region-Republic of Kosova, 1999 to 2014
Fatmire Kollçaku,Shqipe Krasniqi-Kola,Vanina Gafuri,Besa Nila

S9-40 Outbreak of tularaemia in Peja region-Kosovo, January to April 2015
Shqipe Krasniqi-Kola, Fatmire Kollçaku, Adnora Nurboja, Vanina Gafuri

S9-41 see S8-5

S9-42 see S10-1

S9-43 see S10-2

S9-44 see S10-3

S9-45 see S10-4

S9-46 see S11-5

S9-1

Recombinant attenuated *Francisella tularensis* live vaccine strain *capb* mutant overexpressing type VI secretion system proteins induces improved protection against pneumonic tularemia in mice

Qingmei Jia[1], Richard Bowen[2], Barbara Jane Dillon[1] and Marcus A. Horwitz[1]

[1] 37-121 Center for Health Sciences, School of Medicine, University of California – Los Angeles; [2] Department of Biomedical Sciences, Colorado State University [1] Division of Infectious Diseases, Department of Medicine, 37-121 Center for Health Sciences, School of Medicine, University of California – Los Angeles, 10833 Le Conte Avenue, Los Angeles, CA 90095-1688; [2] Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA

A vaccine that is safer and more effective than the currently available unlicensed *Francisella tularensis* Live Vaccine Strain (LVS) is needed to protect against intentional release of aerosolized *F. tularensis,* the most dangerous type of exposure. In previous studies, we developed an LVS Δ*capB* mutant that is significantly safer than LVS yet provides potent protective immunity against virulent *F. tularensis* SchuS4 aerosol challenge; the vaccine is highly protective when administered intranasally, but only partially effective when administered intradermally, and it is less effective than LVS. To improve the immunogenicity and efficacy of LVS Δ*capB*, we developed attenuated recombinant LVS Δ*capB* (rLVS Δ*capB*) strains overexpressing various immunogenic proteins under control of the *F. tularensis* bacterioferritin promoter. The immunogenic proteins, components of a Type VI Secretion System and encoded by *Francisella Pathogenicity Island* genes, include intracellular growth locus A (iglA), iglB, iglC, and a fusion protein (IglA'B'C') comprising immunodominant epitopes of IglA, IglB, and IglC. rLVS Δ*capB* strains express more IglA, IglB, IglC, than the parental LVS Δ*capB* strain in broth culture and in human macrophage-like THP-1 cells (THP-1 cells), grow similarly to the parental LVS Δ*capB* in THP-1 cells, show similar clearance in BALB/c mice, and stably maintain the shuttle plasmids encoding Igl proteins after passage either in THP-1 cells or in mice in the absence of antibiotic selection. Spleen cells from mice immunized intradermally with rLVS Δ*capB* overexpressing IglC or IglA'B'C', after *in vitro* stimulation with IglC or heat-inactivated LVS, secrete interferon-gamma (IFNγ) and interleukin-17 (IL-17) into culture supernatant in amounts greater than spleen cells from mice immunized with parental LVS Δ*capB* and generally comparable to that of spleen cells from LVS-immunized mice. Mice immunized with rLVS Δ*capB* overexpressing IglA, IglB, IglC or IglA'B'C' produce serum IgG, IgG2a and IgG1 antibodies to HI-LVS at levels similar to LVS. Mice immunized intradermally with rLVS Δ*capB* overexpressing IglA or IglA'B'C' and challenged 6 weeks later by aerosol or intranasally with virulent *F. tularensis* SchuS4 survive significantly longer than sham-immunized mice and generally survive longer than mice immunized with LVS Δ*capB*; survival was generally somewhat less than for mice immunized with LVS. rLVS Δ*capB* vaccines overexpressing IglA or IglA'B'C' are stable, immunogenic, induce significant protective immunity, and are safer than LVS. Hence, these vaccines show promise as safe and effective alternatives to the LVS vaccine.

S9-2

Seroprevalence of tularemia among healthy individuals in endemic regions of Georgia

Nino Akhvlediani[1,2], Irma Burjanadze[1], Marina Topuridze[1], Archil Navdarashvili[1], Ciuri Tushishvili[1], David Baliashvili[1], Sopio Dolbadze[1], Mariam broladze[1], Nazi Chitadze[1], Tamar Chikviladze[1], Paata Imnadze[1], Tinatin Kuchuloria[1,2], Tamar Akhvlediani[1,2], Louise-Anne McNutt[3], Robert Rivard[4], Gvantsa Chanturia[1]

National Center for Disease Control and Public Health of Georgia, Tbilisi, Georgia[1]; US Army Medical Research Unit-Georgia, Tbilisi, Georgia[2]; University at Albany, State University of New York, NY, USA[3]; United States Army Medical Research Institute of Infectious, MD, USA[4]

Tularemia is a zoonotic disease endemic in the country of Georgia. Due to low healthcare utilization and lack of laboratory diagnostics, public health officials suspect underreporting of tularemia. In historical epicenters the true prevalence of this infection is suspected to be high due to close proximity of human populations to animals and vectors. To describe the prevalence of antibodies to *F. tularensis* in potentially exposed individuals and to establish risk factors for seropositivity we conducted a seroprevalence study. This effort is part of a broader project funded by the US Defense Threat Reduction Agency (DTRA) to study tularemia in humans, vectors and reservoirs in Georgia. In three regions of Georgia, blood samples were collected in high risk areas. Samples were tested using a microagglutination test (MAT) for antibodies against tularemia. Participants also completed a questionnaire identifying their demographics and possible risk factors for exposure. We report the preliminary results of 364 study participants. The mean age of participants was 45 years (range: 18-67; SD: 13), and 44% are male. The majority of the participants were in the Samtskhe-Javakheti region (62%), followed by Shida Kartli (25%) and Kakheti (13%). Ethnic Georgians comprised 59% of study subjects, followed by ethnic Armenians (26%), and ethnic Azeris (13%). All participants were from rural areas, and were involved in agriculture and animal husbandry. The most common reported animal contact was with dogs and cats (70% and 50%, respectively). Nearly a third (29%) of subjects had contact with rodents. Hunting and fishing was practiced by 9 and 19% of participants, respectively. Most (80%) had contact with hay and wheat, and 24.5% reported being bitten by a tick. One participant was vaccinated against tularemia 25 years ago. The first 229 participants have been tested by MAT revealing 3% (n=10) were positive for tularemia. Eight of the 10 positive samples were male, and none have been diagnosed previously with tularemia. The vaccinated volunteer has not yet been tested. Five of the 10 positive individuals were from the same district of Kaspi in Shida Kartli region, where in 2006, 26 tularemia cases were reported. Otherwise, epidemiological and demographic data of MAT-positive cases were not significantly different from negative cases. This preliminary data provides information about exposure to and risk factors associated with *F. tularensis* in endemic areas of Georgia. The data shows an increased risk of exposure in males residing in Kaspi whose work involves animal husbandry.

S9-3

Extremely successful re-evaluation of Pavlovich's broth medium T for growth of *Francisella tularensis* ssp.

Silke Becker, Petra Lochau, Daniela Jacob, Klaus Heuner, Roland Grunow

Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany

Francisella tularensis (*F.t.*) is a fastidious, relatively slowly growing microorganism which requires special growth conditions. For some applications like testing of antimicrobial susceptibility, isolation from complex matrices, or production of biomass for research and vaccines a liquid growth medium is required. Although some researchers applied for this purpose Mueller-Hinton liquid media, it was fare to being optimal for *F.t.* growth in our hands. N.V. Pavlovich and B.N. Mishankin published in "Antibiotiki i Medicinskaja Biotechnologija", 1987, 2, 133-137, a newly developed transparent growth medium for *F.t.* that is obviously little known in western countries and that we have re-evaluated with an excellent outcome. The basis of broth is a hard-brain extract, casein, casamino acids, glucose, and a number of salts. We used this recipe as Medium T (MT) with and without a selective cocktail of antibiotics and analyzed the growth of various *F.t.* subspecies (*holarctica* LVS and 3 wild isolates, 1 *tularensis*, 1 *novicida*) and other fastidious bacteria. This was compared with bacterial growth in various standard media like Mueller-Hinton II bullion cationic optimized (MH-II), MH-II plus IsoVitaleX (MH-IVX), Bactec Plus Aerobic/F Medium (BP), and Thioglycollate Medium (ThioM). The growth curves based on the calculation of colony forming units (CFU) were obtained under standardized conditions starting with an inoculum of about 100 CFU/ml. The best growth of all bacterial isolates was seen in MT reaching a maximum 10^8-10^9 CFU/ml after 24-36 hrs dependent on the strain. MH-II formulations and BP allowed a much slower growth with a maximum of approx.10^5 CFU/ml except LVS reaching 10^8 CFU/ml after 7 days in MH-IVX. *Novicida* was less dependent of the nature of the medium and reached also very high concentrations in relatively short time. None of the bacterial strains grew in ThioM. In conclusion Medium T comprises an excellent liquid broth medium suitable for enrichment of low numbers of *F.t.* strains freshly isolated or long term cultivated as lab strains.

S9-4

Results on antimicrobial susceptibility testing of *Francisella tularensis* using minimal inhibitory concentration with two methods, gradient diffusion and broth dilution

Daniela Jacob[1], Massimo Fabbi[2], Paola Prati[2], Silke Becker[1], Petra Lochau[1], Roland Grunow[1]

[1] Robert Koch Institute, Centre for Biological Threats and Special Pathogens (ZBS 2), Nordufer 20, 13353 Berlin, Germany; [2] National Reference Laboratory for Tularemia, Istituto Zooprofilattico Sperimentaledella Lombardia e dell'Emilia Romagna (IZSLER), Pavia Department, Italy

Francisella tularensis is the causative agent of tularemia. In Europe only *F. tularensis* ssp. *holarctica* (F.t.h.) was found to cause tularemia in humans so far. The infection is quite often induced through the contact with infected European brown hares (*Lepus europaeus*). Although F.t.h. causes a relative mild form of tularemia in humans, an adequate antibiotic treatment is indispensable. Among the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) no recommendations of minimal inhibitory concentration (MIC) testing and interpretation of data on *F. tularensis* are given. For the further evaluation of protocols for Antimicrobial Susceptibility Testing developed in the framework of the Joint Action "Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens" (QUANDHIP), we used 16 isolates of F.t.h. collected from 1964 until 2010 mostly from hares. The developed standard operation procedure (SOP) considers the special requirements for growth of these fastidious bacteria. The MIC was tested by gradient diffusion using Liofilchem stripes and broth microdilution on commercial test plates from Merlin, MICRONAUT, comprising a panel of 12 freeze-dried antimicrobial agents (penicillin, vancomycin, clindamycine, imipenem, ciprofloxacin, levofloxacin, gentamicin, tigecycline, doxycycline, rifampicin, chloramphenicol, and linezolid). The results of both methods were compared and the MIC data were interpreted according to the American Clinical and Laboratory Standards Institute (CLSI) criteria, when available. Both methods showed a good performance and are applicable for highly infectious fastidious bacteria under appropriate biosafety conditions.

S9-5

Meropenem resistance in the MRIGlobal *Francisella tularensis* repository

Robyn Kelly, Sara Hamilton, Deandra Audley, Aaron Tyler, H. Carl Gelhaus

MRIGlobal, 425 Volker Blvd., Kansas City, MO, USA

Background Antibiotic resistance is an area of significant drug development and repurposing of broad spectrum antibiotics with primary indications for multi-drug resistant bacterial infections is one method to reduce costs for a secondary biodefense indication. Carbapenem resistance is of particular interest since drugs in this class, such as meropenem, are often considered “drugs of last resort,” used only after other antibiotic choices have been shown to be ineffective. Given the intense interest in carbapenem resistance, repurposing carbapenem resistant infections therapies to include biodefense indication may benefit drug developers as well as the U.S. government. This requires the use of a carbapenem-resistant biodefense pathogen. Therefore, we studied meropenem-resistance in *F. tularensis*, a Tier 1 select agent. Antibiotic susceptibility studies of less virulent *F. tularensis* subspecies holartica strains isolated from patients and hares found that 47 of 50 strains were resistant to meropenem. It was hypothesized that some of the highly virulent *F. tularensis* subspecies *tularensis* strains at MRIGlobal contained meropenem resistant. In order test this hypothesis, minimal inhibitory concentrations (MIC) of meropenem for the six *F. tularensis* strains in our collection were determined. ESKAPE pathogen *Eschericia coli* and multi-drug resistant/ methicillin resistant *Staphylococcus aureus* (MRSA) were also tested as quality controls. We found that *F. tularensis* strains, as well as the MRSA strain, were highly meropenem resistant.

Methods Six strains of *F. tularensis*, including fully virulent type A strains and clinical isolates, were tested at least three times by ETest®. Two technical replicates were performed for each test, giving a total of at least six MIC values for meropenem in *F. tularensis*. All plates were incubated at 37°C for 1-4 days, until bacterial growth was complete. The MIC reported for each strain was taken from the mode of all recorded values.

Preliminary Results An MIC of >32 µg/mL was observed for all six *F. tularensis* strains had, indicating resistance.

Preliminary Conclusions MRIGlobal’s collection of virulent *F. tularensis* contains several strains that are highly resistant to meropenem. These strains are ideal to study carbapenem resistance in BWA, both in vitro and in

S9-6

Experimental basis of rational treatment of tularemia

N.V. Pavlovich, M.V. Tsimbalistova

Rostov-on-Don Research Institute for Plague Control, Rostov-on-Don, st. M. Gorkogo 117, 344002, Russia

In the last decades the threat of using *Francisella tularensis* as bioterrorism agent is increasing. That is why the problem of prophylaxis and treatment of tularemia acquires special significance. It is important to note the possibility of construction of antibiotic resistant strains which may be used for bioaggression aims. *F. tularensis* is divided into three subspecies. Human infection is mainly caused by two subspecies – tularensis and holarctica. However *F. tularensis* subsp. *mediasiatica* induces infection in humans and rabbits more severe than holarctica strains (Koturga L et al., 1981). For this reason the development of effective treatment of tularemia must include strains of three main subspecies. *F. tularensis* is characterized by natural resistance to β-Lactams, macrolides, polymyxin and dalacin. Earlier, we showed that resistance to penicillins and cephalosporins is formed by two different mechanisms – first (β-Lactamase) and second (impermeability of cell wall for antibiotic) (Pavlovich N. et al.,1992; Tsimbalistova M., Pavlovich N., 2014). In our work we studied efficiency of various antibiotics including cephalosporins against tularemia in murine model (infective dose - 1000 DCL) caused by various strains of three subspecies, the elimination of microbes after treatment and possibility of the induction of specific immunity after therapy. Aminoglycosides (streptomycin, gentamycin, amicacin), fluoroquinolones (ciprofloxacin, levofloxacin, lomefloxacin, moxifloxacin) and rifampicin were active against *F. tularensis* but their efficiency was dependent on personal characteristics of infecting strain. So, the percent of survived animals ranged from 30 to 100%. Doxycycline, chloramphenicol, fortum, cefotaxime, amoxiclav and tienam were characterized by low efficiency (no more than 30% of survivors).The investigation of the formation of the immunity against tularemia (specific antibodies, reinfection of surviving mice with virulent strain) after etiotropic therapy showed that effective antibiotics caused immunosuppression and did not induce the specific immunity. Interestingly, when using effective antibiotics (aminoglycosides, fluoroquinolones) we isolated the culture of the pathogen from the surviving mice. Therefore, tularemia treatment by antibiotics did not ensure the full elimination of microbes, which creates a high risk of late relapses of the disease. Combination of fluoroquinolones+rifampicin or aminoglycosides+rifampicin resulted in high efficiency of treatment (independent of infecting strain) and full clearance of mice from tularemia agent. Thus, in cases of severe human infection supposed to be the tularemia it is reasonable to carry out the combined antibiotic therapy which should improve the efficiency of treatment and decrease the risk of relapses.

S9-7

Entry of *Francisella tularensis* into B cells

Lenka Plzakova, Klara Kubelkova, Zuzana Krocova, Ales Macela

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, 1575 Trebesska, 500 01 Hradec Kralove, Czech Republic

Francisella tularensis, the causative agent of tularemia and a potential biowarfare agent, is a facultative intracellular pathogen causing zoonotic disease in a wide variety of species. Both *in vitro* and *in vivo*, *Francisella* spp. infect and proliferate inside phagocytic cell types. *Francisella tularensis* also infects non-phagocytic hepatocytes, epithelial cells, and B cell lines. Information available about immune response to *Francisella* spp. comes from studies on natural human infections or immunizations as well as from animal model studies. *Francisella tularensis* enters into B cells. B cells have a wide range of functions within the immune response, including recognition of antigens, antigen presentation to competent T cells, production of cytokines and antibodies, and contribution to the development of immunological memory. The entrance of *F. tularensis* into B cells requires both the active participation of bacteria and engagement of the B cell receptor and the other receptors we analyzed. Entrance of the bacteria into B cells occurs through ligation of B cell receptor and complement receptor 1/2. Our experiments have shown that B cell receptor and complement receptor 1/2 are involved in *ex vivo* recognition and engulfment of *Francisellae* into B cells. The complement receptors 3 and 4 and the FcγR receptor are not involved in these processes. The effect of entry of the blocking B cell receptor on CD19+ cells or separate B cell subsets demonstrated that B cell receptor alone is sufficient for the entry of *F. tularensis* into B-1a cells only. Intracellular trafficking of *F. tularensis* inside B cells is distinct from intracellular trafficking within other antigen-presenting cells. Once inside phagocytic cells, *Francisellae* escape from the phagosome into the cytosol where they proliferate. On the other hand, living *Francisellae* can be localized in the membrane surrounding the vacuole inside B cells. They do not proliferate there. The trafficking of *F. tularensis* was determined by detecting colocalization of the bacteria with the early endosome antigen EEA-1, late endosomal/lysosomal membrane marker LAMP-1, and cathepsin D.

This work was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense of Czech Republic and Grant No. P302-11-1631 from the Czech Science Foundation.

S9-8

Characterisation and exploitation of RelA as a novel antibacterial target for the biowarfare agent *Francisella tularensis*

Rachael C. Wilkinson, Petra C.F. Oyston, and Peter L. Roach

Department of Chemistry, University of Southampton[1], DSTL, Porton Down[2]
Department of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK

Recent years have seen a rise in bacterial resistance to clinically available antibiotics in tandem with a decrease in the discovery of novel antimicrobials. A key outcome of this is the renewed threat posed by both public health pathogens and biowarfare agents, for which treatment has previously been available. One way to circumvent this issue is by the identification of novel targets to allow the development of new antimicrobials. Such work requires preliminary understanding and characterization of the novel target.
In bacteria the stringent response coordinates several metabolic pathways to facilitate survival in unfavourable conditions. This intrinsic response exploits the global regulator (p)ppGpp, the production of which in β- and γ-Proteobacteria is predominantly catalyzed by the enzyme RelA. Knockouts of this enzyme have been shown to reduce the virulence of pathogenic bacterial species, including a model organism for the biowarfare agent *Francisella tularensis*.

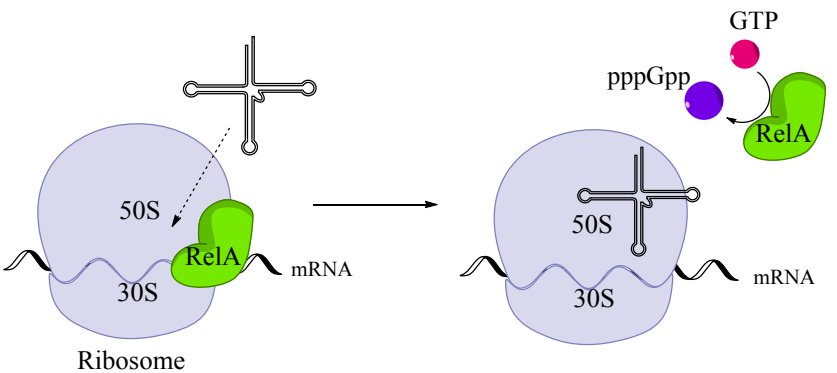

Figure 1 - Activation of RelA by a stalled ribosomal complex, formed by the entry of unacylated tRNA into the ribosomal A site.

The enzyme RelA undergoes complex activation, figure 1, and further understanding of the homolog from *Francisella tularensis* is required for future development of inhibitors. Kinetic parameters for the enzyme were derived by both HPLC and ^{31}P NMR activity assays. Activation of the homolog by both ribosomes and ppGpp

was demonstrated and an EC_{50} for ppGpp derived. Further work has led to the development of a novel high throughput screen for RelA demonstrated by the use of the *F. tularensis* homolog. The high throughput screen has been validated using focused small nucleotide analog libraries. Collectively this work has elucidated a further understanding of the RelA homolog from *Francisella* and has derived a method capable of identifying potential new antibacterial compounds for this organism.

S9-9

Immunodiagnostic and molecular methods in a twelve-year follow-up of patients suffering from tularemia in Serbia

Marina Djordjevic-Spasic[1], Herbert Tomaso[2]

[1] Infectious Diseases Clinic, Clinical Centre, University of Nis, Serbia; [2] Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena, Germany

Introduction: Tularemia is a severe bacterial zoonosis caused by the highly infectious agent *Francisella tularensis*. Microbiological diagnosis of tularemia mainly relies on serology. Occurance of tularemia epidemic in the Southeast of Serbia in 1998/1999. initiated epidemiological as well as microbiological and clinical research in this area in the next ten years. That was the motive to start applying and improving immunodiagnostic tests, and a real challenge for the clinicians to fight the infectious agents, bacteria *F. tularensis*.

Objective was a twelve-year follow-up of patients suffering from tularemia in Sounth-Eeastern Serbia from 1999. till 2011.

1. Monitoring and evaluation of the serological antibody finding of tularemia patients within the period from 1 to 11 years since the beginning of the disease.
2. Determination of the correlation between the test results done by immunodignostic, histological, immunohistochemical and molecular methods; significance defining of the noted tests in diagnosis and the efficiency assesment in therapy of tularemia patients.
3. Efficiency assesment of the applied antibiotic therapy in patients suffering from tularemia.

Methods: Testing was performed on a group of 113 patients suffering from tularemia in a period starting in 1999. until the end of 2011. Control group included 111 examinees, out of which 31 patient with differential diagnosis of lymphadenopathy of different origin, 20 seropositive brucellosis patients, 10 seronegative non-brucellosis patients, 30 seropositive yersiniosis patients and 20 voluntary blood donors.

During the diagnostic processing of patients, following materials were used: serum and bioptat of lymph node. Total of 270 serum samples were examined, out of which 189 serums suspicious of tularemia, 111 serums from the control group and 26 lymph node bioptats.

There were 8 diagnostic techniques used on the material mentioned, out of which 6 serological, immunohistochemical method of indirect immunoperoxidase (IIP) and molecular method (PCR). Serological methods included immunoensyme assays: Serion ELISA IgG i IgM, Serazym ELISA and ELISA in-house; immunochromatographic test (ICT) and Western blot (WB). On patohistological material, IIP was done with the use of primary monoclonal antibodies on *F. tularensis*, and the molecular method done, was PCR.

Results: Serums of the patients monitored in the interval of 1 to 11 years starting with the occurrence of the disease, indicated positive finding of IgG, IgM class antibodies, as well as total antibodies on *F. tularensis,* in most of the tests. Analysis of the results obtained in the used tests, confirmed that most of the tests indicate high sensitivity, specificity and mutually similar results. IgG Serion test showed the highest sensitivity (97,4%) and specificity (93,1%). IHH method of the indirect immunoperoxidase (IIP) proved the highest specificity (100%), but low sensitivity (65%). Complications were noticed in 43,4% patients mostly with suppurative lymphadenitis and recidivism of the disease. Two thirds of the complications occurred after late onset of appropriate treatment, 3 weeks after the beginning of illness. The highest percentage of healing and the least complications were noted with the use of gentamycin (83,3%), then ciprofloxacin (75%) and finally, in the successive therapy with gentamycin and doxycycline (70,0%). The biggest failure in children patients was noted in the use of amikacin with ceftriaxone which is related to occurance of suppurrative lymphadenitis in all treated children.

Conclusion: The use of immunodiagnostic tests confirmed the old *F. tularensis* infection and established retrograde diagnosis on previously undiagnosed or serologically unconfirmed tularemia. Serological finding must be interpreted only within clinical diagnosis of tularemia. Finding of IgM and IgG class antibodies or total antibodies on *F.tularensis* in serums of the patients without clinical manifestations of the disease, even many years after the beginning of the infection, does not indicate acute illness, but the cured illness, which once existed. Test which proved highest sensitivity and specificity is IgG Virion ELISA, therefore it is suggested as the most reliable method for diagnosis and monitoring of tularemia patients. Immunohistochemical method IIP can be used in confirmative diagnosis of tularemia due to its highest specificity. PCR method is not a method of choice for diagnosis of tularemia on biopsy samples of the tissue moulded in paraffin. The success of therapy was proved in healing of all patients monitored within the period from 1 to 11 years (96,5% of the patients) except for the persistent lymphadentopathy in 4 patients (3,5%). There was no influence of the therapy on results of patients' serological analysis.

S9-10

Effect of Fe-superoxide dismutase activity on the biological properties of the *Francisella tularensis* 15 vaccine strain

M.A. Sukhova, T.B. Kravchenko, A.N. Mokrievich, V.M. Pavlov

State Research Scientific Center for Applied Microbiology and Biotechnology 142279, Moscow reg., Serpukhov distr., Obolensk; info@obolensk.org. +7(4967)36-00-10, Russia

Francisella tularensis is the causative agent of tularemia. This microorganism has a group of superoxide dismutase enzymes for bacteria survival in the host cells. Fe-superoxide dismutase (FeSOD) is one of the group. FeSOD allows not only to avoid damaging of cells by free radicals formed during the metabolism of bacteria, but also protect bacteria from environmental superoxide radicals. We investigated the effect of FeSOD activity on *F. tularensis* growth in liquid medium. It is shown that the level of synthesis of FeSOD was decreased during multiplication of microbe variants in which *sodB* native codons were substituted on rarely codons for genome of tularemia microbe: start codon ATG for GTG, TTA (Leu) for TTG (Leu) and CCA (Pro) for CCC (Pro). Nucleotide substitution *sodB* gene was made by allelic exchange using the suicide plasmid pGM5. As the result three types of clones with the substitution in *sodB* gene was obtained: SodBI (replacement in the start codon), SodBII (replacement in the start and leucine codons) and SodBIII (replacement in the start and proline codons). According native electrophoresis the level of FeSOD expression in logarithmic growth phase of *F.tularensis* decreased by 25-30 % for strain SodBI and by 40-50 % for strains SodBII and SodBIII, but in stationary growth phase the level of expression was similar for all strains. This indicates that in the process of multiplication of bacteria the FeSOD concentration is decreased. Addition of hydrogen peroxide in concentrations 7,5 mM into liquid microbe culture in the logarithmic growth phase resulted in growth inhibition of strains SodBI, SodBII, SodBIII compared to parental strain, however without hydrogen peroxide strains were growing with the same speed. Exposure of *F. tularensis* modified strains to hydrogen peroxide has led to some increased synthesis of mRNA of *groEL* gene in all strains, including the parent, but only in SodBII and SodBIII strains there was a significant increase of the mRNA than in the parent strain. This strains are interesting for studying the effect of FeSOD activity at the immunogenicity of tularemia microbe. Also using *sodB* as a example we confirmed the possibility reducing the of genes expression level in *F. tularensis* 15 vaccine strain by substitution of native codons on rarely in a tularemia microbe genome.

S9-11

Analysis of *Francisella*-immune murine peripheral blood leukocytes reveals new insights in the identification of potential correlates of protection against *Francisella tularensis*

Lara Mittereder, Roberto De Pascalis, Nikki J. Kennett and Karen L. Elkins

Laboratory of Mucosal Pathogens and Cellular Immunology, DBPAP / OVRR / CBER / FDA, 10903 New Hampshire Avenue, Building 52/72, Room 5330, Silver Spring, MD, USA 20993

Peripheral blood leucocytes (PBLs) represent an easily accessible source of immune cells from humans, and therefore the best source to investigate cell-mediated immune responses from infected or vaccinated people. Although human clinical trials of vaccines against tularemia are impractical due to the sporadic nature of disease, preclinical studies may be bridged from animals to humans by taking advantage of correlates of protection. Previously, using splenocytes from C57BL/6J mice vaccinated with *F. tularensis* LVS-derived vaccines, we identified and quantified correlative T cell immune responses that discriminated vaccines of different efficacy. In addition, we demonstrated that the relative levels of gene expression of potential correlates of protection varied according to vaccination route, and between cell types from different organs. To evaluate whether our approach to identify correlates of protection also applies to an accessible source of immune cells, here we extended our studies to the analysis of mouse PBLs. Although immune murine PBL cell populations differed slightly from splenocytes, overall the *in vitro* functions, such as control of LVS intramacrophage replication, IFN-γ production, and nitric oxide production, reflected the hierarchy of LVS-derived vaccines protection and appeared similar between splenocytes and PBLs. Selected potential correlates of protection, which were previously identified by using co-culture of LVS-immune splenocytes, were also differentially expressed in immune PBLs. Further, a more extensive screening of immune response-related genes using PBLs identified additional potential correlates of protection, such as IL-21, IL-2RA, granzyme, CCL5, Fas ligand, and LTA. In contrast, analyses of secreted proteins in supernatants derived from the *in vitro* co-cultures revealed minimal differences between PBL samples from differentially vaccinated mice. Finally, the expression of selected identified genes was different among vaccine groups in PBLs obtained directly from mice shortly after primary vaccination, without *ex vivo* restimulation. The results not only suggest that these cells are involved in the immune response, but also that their gene expression may represent a tool to monitor efficacious immune responses against *F. tularensis* vaccination.

S9-12

Data mining and genetic studies of *Francisella tularensis* in search of novel tularemia vaccine candidates

Galia Zaide, Naomi Ariel, Itzhak Inbar, Uri Elia, Hila Cohen, Gila Friedman, Anat Zvi, Avigdor Shafferman and Ofer Cohen

Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, P.O.Box 19, Ness Ziona, 74100, Israel.

F. tularensis is a Gram-negative, intracellular pathogen representing the etiological agent of Tularemia, a relatively rare disease in the Western world, efficiently treatable by prompt antibiotic administration. Owing to the high inhalational infectivity, ease of dissemination and lethality of Tularemia, *F. tularensis* is designated by the CDC as a category A select agent. As of today, there is no licensed Tularemia vaccine and consequently the development of an effective vaccine is of high priority in the context of countermeasure strategies. Identification of targets for development of live attenuated vaccines against *F. tularensis* is particularly challenging since it lacks close pathogenic relatives and it does not encode toxins or secretion systems commonly present in other intracellular pathogens. Extensive data mining of reported global analyses involving various *Francisella* strains was implemented for selection of vaccine candidates against Tularemia. A ranking/prioritization algorithm was applied for assignment of quantitative scores to virulence-related antigens, immunoreactivity and protection, resulting in identification of 40 antigens possibly involved in pathogenicity and consequently putative vaccine candidates. Eight LVS insertion mutants disrupted in ORFs encoding the top ranking antigens were constructed and their impact on virulence was determined. Six of these mutants exhibited significant MTTD increase; Δ*htpG* and Δ*atpC* mutants exhibited an attenuated phenotype manifested by 20 fold increase in LD_{50} value as compared to the parental LVS strain and conferred robust protection against lethal challenge of LVS. Most notably, a Δ*groEL* mutant showed a significant decrease in virulence both by I.P and I.N route of infection.

S9-13

Synthetic bis-indolic derivatives with antibacterial activity against *Francisella tularensis* subspecies *holarctica*

Yvan Caspar[1,2#], Jean-Noël Denis[3], Frédéric Minassian[3], Max Maurin[1,2]

[1] Laboratoire de bactériologie, Institut de Biologie et Pathologie, CHU de Grenoble, [2] LAPM, UMR-5163 CNRS, Université Joseph Fourier Grenoble-1, BP 170, Grenoble Cedex 9, France, [3] DCM, UMR-5250 CNRS, Université Joseph Fourier, BP53, 38041, Grenoble Cedex 9, France

Antibiotic classes currently recommended to treat tularemia include the aminoglycosides (gentamicin and streptomicin), fluoroquinolones (ciprofloxacin) and tetracyclines (doxycycline). However, despite administration of an appropriate antibiotic therapy, treatment failures and relapses remain frequent, especially when treatment is delayed. Moreover, selection of resistant *F. tularensis* strains to these antibiotics can be easily selected *in vitro*. Thus, the search for new therapeutic alternatives active against this highly virulent human pathogen is still necessary. In this study, we evaluated novel synthetic bis-indolic compounds against *F. tularensis* subsp. *holarctica*. Minimal Inhibitory Concentrations (MICs) of 4 bis-indolic compounds (dcm01, dcm02, dcm03 and dcm04) were determined using a broth microdilution method according to CLSI guidelines, against 41 clinical strains (Ft1 to Ft41) of *F. tularensis* subsp. *holarctica* isolated in France between 2006 and 2013 and identified at the National Reference Center of Tularemia, Grenoble, France. Reference strains of *F. tularensis* subsp. *novicida* CIP56.12, *F. philomiragia* ATCC25015 and the attenuated *F. tularensis* subsp. *holarctica* LVS strain were also included in our assay. Minimal Bactericidal Concentrations (MBCs) were determined for lead compounds dcm02 and dcm04 for three strains (Ft6, Ft24 and LVS). Killing curves were measured for these three strains exposed to dcm04. The MIC_{90} was 8 µg/ml for dcm01, dcm02 and dcm03, and 2 µg/ml for dcm04. All *F. tularensis* subsp. *holarctica* strains tested were susceptible to dcm02 and dcm04, with MICs ranging from 2 to 8 µg/ml. Only one strain (Ft5) was resistant to both dcm01 and dcm03, with MICs > 32 µg/ml. Such variations in antibiotic activity may be explained by structural differences between the compounds, especially the presence of a large CH2NHBoc chemical group in dcm01 and dcm03, whereas it is replaced by a methyl group in dcm02 and dcm04. *F. tularensis* subsp. *novicida* reference strain was resistant to all 4 derivatives with MICs ≥ 32 µg/ml. *F. philomiragia* strain was only susceptible to dcm02 and dcm04 with MIC of 16 and 4 µg/ml, respectively. A bactericidal activity of dcm02 and dcm04 was observed against attenuated LVS strain, whereas these compounds were only bacteriostatic against Ft6 and Ft24 strains. In conclusion, we have identified novel synthetic bis-indolic compounds, active against *F. tularensis* subsp. *holarctica* but not the closely related *F. tularensis* subsp. *novicida* strains. These compounds may represent drug candidates for the development of new therapeutic alternatives for tularemia treatment. The bacterial targets of these new compounds remain to be characterized.

S9-14

Vaccine-mediated protection against *Francisella tularensis*

Igor Golovliov, Kjell Eneslätt, Helena Lindgren, and Anders Sjöstedt

Department of Clinical Microbiology, Umeå University, Umeå, Sweden

Despite decades of research, a tularemia vaccine has not been licensed. The currently used human vaccine, the LVS strain, is not licensed for general use since it has obvious shortcomings as it was empirically derived and confers incomplete protection against aerosol challenge. We have identified a deletion mutant of the highly virulent SCHU S4 strain lacking the *clpB* gene (Δ*clpB*) as a live vaccine candidate. It is more efficacious and less reactogenic than the LVS strain and confers protection against aerosol challenge even with strains of the highly virulent ssp. *tularensis* (type A) in mice. In view of the potential of Δ*clpB* as a vaccine candidate, the present study was aimed to identify why it confers superior efficacy compared to the LVS strain. Effector mechanisms of splenocytes of Δ*clpB*- or LVS-immunized mice were investigated using an *in vitro* model. One to six months after immunization, splenocytes were obtained from immune or naïve mice and added to bone marrow-derived macrophages infected with the SCHU S4 or LVS strain. There was net growth in the cultures over a period of 3 days, however, in the presence of Δ*clpB*- or LVS-immune splenocytes, control was very significant compared to naïve splenocytes. Neutralization of IFN-γ or TNF-∝ in the cell cultures with Δ*clpB*- or LVS-immune splenocytes significantly reduced the control of bacterial replication, although the control still was significantly better than in the presence of naïve splenocytes. Correlations between control of bacterial replication and levels of nitrite, an end product of nitric oxide reactivity, and cytokines moreover, were assessed. Corroborating the important role of nitric oxide, addition of NMMLA, a competitive inhibitor of iNOS, significantly reduced the control of bacterial replication and no control was observed when BMDM from $iNOS^{-/-}$ mice were infected with the SCHU S4 or LVS strain. Wild type macrophages co-cultured with Δ*clpB*-immune splenocytes consistently produced higher levels of either IFN-γ or nitric oxide than did macrophages co-cultured with LVS-immune splenocytes. The results identify a critical role of nitric oxide for control of *F. tularensis* replication in the *in vitro* model and also demonstrate that the Δ*clpB*-immune splenocytes lead to higher levels of nitric oxide than does LVS-immune splenocytes.

S9-15

Limited potential of highly virulent *F. tularensis* strain to induce protective immunity against *F. tularensis* challenge

Erez Bar-Haim

Biochemistry and Molecular genetics Department, The Israel Institute for Biological Research, Israel

The Live Vaccine Strain (LVS) serve as the prototype for live vaccines against *F. tularensis*. Currently, most of the live vaccines under development are based on LVS, or on genetically-modified *F. tularensis* strains either LVS or the more virulent strain SchuS4. We established a novel murine experimental system that facilitates assessment of the protective potential of LVS, compared to the highly virulent SchuS4 strain. This is achieved by infection with lethal doses of either strain, followed by effective antibiotic treatment. To attain a comparable level of vaccination, mice were infected with 10^5cfu of LVS or 10^2cfu of SchuS4. Seventy two hours later, bacterial loads in reticuloendothelial organs of mice from both groups were comparable. At this time point ciprofloxacin treatment was initiated, to achieve complete elimination of the infecting bacteria. Five weeks post infection, vaccinated mice were challenged with lethal doses of LVS or SchuS4 strains. SchuS4-primed mice did not survive homologous challenge with SchuS4 bacteria, and were only partially protected from the heterologous challenge with LVS strain. Conversely, LVS-primed mice exhibited superior resistance to challenge with SchuS4 bacteria than Schu-primed mice. Co-infection vaccination experiments with both strains demonstrated protective response pattern equivalent to the LVS-vaccinated mice, indicating that SchuS4 bacteria did not induce an active systemic immune suppression. Various Immune analyses indicate that the limited protective response of SchuS4-vaccinated mice is not a result of different quantity of responding T cells, as demonstrated during the priming process and before challenge. Furthermore, the limited protective value of the SchuS4 strain is not correlated with the production and secretion of various cytokines. Possible mechanisms for strain-dependent protection will be discussed. Our data demonstrate that the protective outcome of live vaccines may depend on the vaccinating strain, and it should represent a significant parameter for development of genetically-modified, SchuS4-based strains as live vaccines.

S9-16

A poly-epitope DNA vaccine engineered on the basis of *Francisella tularensis* whole-genome immunoinformatic analysis elicits a protective CTL immune response

S. Rotem

IIBR, Ness-Ziona, Israel

The cellular arm of the immune response plays a central role in the defense against intracellular pathogens, such as *Francisella tularensis*, the causing agent of Tularemia. State-of-the-art prediction methods developed in recent years enable to map MHC class I binders which might stimulate the CD8+ mediated immune response. Nevertheless, the number of predicted peptide candidates for experimental evaluation is still in the range of ten-thousands, even for a limited coverage of MHC alleles. We have recently developed an unbiased, whole-genome approach for rational selection of putative CTL epitopes and enrichment of true responders, based on highly dense “hotspots” of predicted MHC class I binders. Application of this approach to the proteome of *F. tularensis* (LVS strain) resulted in selection of a library of 2800 putative CTL epitopes which were tested for their ability to stimulate IFNγ secretion from splenocytes isolated from LVS vaccinated C57BL/6 and BALB/c mice. Altogether, over 220 novel epitopes were identified, increasing by over 50% the dataset of known T-cell epitopes of *F. tularensis*. A DNA vaccine expressing six of the most potent identified CTL epitopes (based on number of CD8+ T cells secreting IFNγ) was shown to elicit specific CD8+ T cell responses to all encoded epitopes, devoid of an observed CD4+ T cell response. Moreover, this CTL-mediated immune response can confer protection against lethal systemic as well as a lethal pulmonary challenge of *F. tularensis* LVS. Finally, our novel approach for an unbiased whole genome identification of CTL epitopes may be applied to the development of CD8-epitopes driven vaccines against other complex intracellular pathogens.

S9-17

Molecular evidences of *Francisella tularensis* in rodents in Iran

Abdolrazagh Hashemi Shahraki[1,2], Ehsan Mostafavi[1,2], Ahmad Ghasemi[1, 2], Ahmad Mohamoudi[3], Jamshid Darvish[3], Mansour Aliabadian[3]

[1] Department of Epidemiology, Pasteur Institute of Iran, Tehran, Iran; [2] National reference laboratory for Plague, Tularaemia and Q fever, Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Akanlu, Kabudar Ahang, Hamadan, Iran; [3] Rodentology Research Department, Applied Animal Institute, Ferdowsi University of Mashhad, Mashhad

Background and aim: Tularemia disease is caused by the bacterium *Francisella tularensis*. Our understanding of the life cycle of the organism in nature and it's natural reservoirs is still limited in Iran. The main objective of this study was to obtain information about the occurrence of *F. tularensis* in rodents in Iran.
Material and methods: During 2014, 96 spleen samples were collected from 96 different species of hunted rodents around Iran. Rodents were hunted from 15 locations of seven different provinces in Iran including Fars, Chaharmahal and Bakhtiari, Goegan, Kermanshah Khuzestan, Zanjan and North Khorasan. After DNA extraction, the samples were subjected to Real time PCR for detection of *F. tularensis*
Results: Hunted Rodents were 24 *Microtus paradoxus*, 15 *Microtus irani*, 13 *Microtus socialis*, 13 *Mus musculus*, 10 *Apodemus witherbyi*, 6 *Crocidura suaveolens*, 3 *Calomyscus bailwardi*, 3 *Mus macedonicus*, 3 *Microtus qazvinensis*, 2 *Meriones persicus*, 2 *Neomys anumalus*, 1 *Microtus mystacinus* and 1 *Rattus rattus*.
Real-time PCR screening demonstrated the presence of *F. tularensis tul4* and *fopA* genes in one rodent (1%), *Microtus paradoxus* in Gorgan province, northern Iran.
Conclusion: Our results indicate that *Microtus paradoxus* can carry *F. tularensis*. Thus, they may have a role in the ecological cycle of this pathogen. This finding confirmed the presence of *F. tularensis* among the rodent's populations in Iran. Further serological and molecular surveys in rodents are needed to reveal pathogen dynamics in rodent populations in Iran.

S9-18

Serological survey of tularemia in rodents in western Iran

E. Mostafavi[1,2], A. Hashemi Shahraki[1,2], AR. Japoni-Nejad[1], S. Esmaeili[1,2], MM. Gouya[3], J. Darvish[4], MM. Sedaghat[5], A. Mohammadi[5], ZA. Mohammadi[4], A. Mahmoudi[4], B. Pourhossein[1,2], M. Gyuranecz[6]

[1] Department of Epidemiology, Pasteur Institute of Iran; [2] Director of the Research Centre for Emerging and Reemerging infectious diseases (National Reference center for diagnosis and research on Plague, Tularemia and Q fever), Pasteur Institute of Iran, Akanlu, Kabudar Ahang, Hamadan, Iran; [3] Centre of Disease Control (CDC), Ministry of Health; [4] Rodentology Research Department, Applied Animal Institute, Ferdowsi University of Mashhad, Mashhad; [5] Faculty of Health, Tehran University of Medical Sciences, Tehran, Iran; [6] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences Budapest, Hungary

Background: *Francisella tularensis* is the causative agents of tularemia. Tularemia has the potential to have high morbidity and mortality. Since the Kurdistan province in the western region of Iran has been the historical foci of tularemia, the aim of this study was to evaluate the current situation of the rodents living in this region regarding the tularemia.
Material and Methods: In this study, conducted in 2014 in five districts; Marivan, Saghez, Sarvabad and Sanandaj in Kurdistan province and Sonqor in Kermanshah province, the rodents were captured by live traps. The ectoparasites, spleen and serum samples were collected from the rodents. Genus and species of the rodents and their ectoparasites were identified. Serological survey of tularemia was performed by standard tube agglutination tests. Real Time PCR was used for detection of *Francisella tularensis* DNA.
Results: Of 246 captured rodents, the more frequent rodents were from the genus Apodemus (39.84%), Mus (24.39%) and Meriones (12.60%). 78 fleas, 73 mites and 14 ticks were collected from the studied rodents. Seroprevalence of tularemia in the studied rodents was 4.8% (8/164) with 1/80 or higher antibody titers. The suspected tularemia seropositive rodents (1/40 antibody titers) were identified in nine cases. The results of microscopic examination and Real Time PCR were negative for all the samples.
Discussion: This is the first report of tularemia infection in rodents in Iran. Further study of the rodents and the environmental samples such as water can further clarify the epidemiological aspects of tularemia in the future.

S9-19

Whole-genome sequencing of Italian isolates of *Francisella tularensis* subsp. *holarctica*: preliminary data

Nadia Vicari[1], Erika Scaltriti[2], Chiara Chiapponi[2], Carmen Mandalari[2], Andrea Manfredini[1], Stefano Pongolini[2], Massimo Fabbi[1]

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER): [1] National Reference Laboratory for Tularemia, Pavia Department (Italy); [2] Parma Department (Italy) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), Sezione di Pavia, Strada Campeggi, 59/61 – 27100 Pavia, phone: +39 0382422006, fax: +39 0382 422066, Italy.

Tularemia is a zoonosis caused by *Francisella tularensis.* The bacterium infects lots of vertebrates and is either transmitted directly through close contact with infected animals (tissues and body fluids) and via insect bites, or indirectly through inhalation/ingestion of contaminated aerosols, water and food. Currently only *Francisella tularensis* subsp. *holartctica* (F.t.h.) has been detected in Europe. In Italy the first confirmed cases of tularemia date back in 1964 when the disease was detected in European brown hares and humans. Since then, very rare outbreaks or sporadic cases of tularemia occurred, but infected hares have been detected almost every year. We performed whole-genome sequencing of 35 Italian isolates of F.t.h. recovered between 1964 and 2015 from different sources (animals, human and water). Our aim was to understand the genetic relationships among them and European isolates. The genomes were sequenced by MiSeq Illumina system and *de-novo* assembled. Core SNPs were identified with a MAUVE-based approach and used for phylogenetic studies with MrBayes and Beast softwares. Bayesian tree highlights that Italian field isolates are divided into two main clusters, one cluster encloses isolates from imported hares (East Europe n=16) and the other one includes isolates recovered from autochthonous hares (n=16), natural spring water (n=2) and human patient (n=1). Notably, strains belonging to this last cluster are very similar to West Europe isolates. These results confirm previous data obtained by MLVA subtyping carried out on the same isolates. Furthermore, molecular clock analysis suggests some possible dates for lineages separation.

S9-20

Study of the biological threats for *F. tularensis* distribution in environmental objects

B.T. Stegniy, A.P. Gerilovych, A.I. Zavgorodniy, O.V. Obykhovska, V.L. Arefiev

National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine, antger@vet.kharkov.ua

Background: Tularemia is caused by *Francisella tularensis*. Different clinical forms could characterize the disease. Due to its extreme infectivity and ability to cause disease upon inhalation, *F. tularensis* has been classified as a biothreat agent. The different ways of disease distribution are described. The rodents and ticks could play potential role in the disease spread. This study aimed to perform PCR-based screening of the environmental specimens, potentially could be contaminated by F.tularensis.
Methods: Sampling has been managed in Eastern (Kharkov, Sumy) and Southern (Odessa and Mykolaiv regions) Ukraine. The soil, dust, samples of the feedstuff, rodent feces, ticks were collected from 14 farms and 18 backyards using traditional techniques. DNA extraction from the samples has been managed using CTAB-based deep destruction, sorption on the liquid silica and elution to the TE-buffer. PCR has been carried out by O.I.E. recommended protocol.
Results: The screening of the environmental objects, i.e. samples of soil (n = 49), dust from the animal and feedstuff storage facilities (n = 24), and feedstuff (n = 51) demonstrated the absence of *F. tularensis* DNA. The same results were observed during the screening of tick-derived samples (n = 38), collected from animals and in the environment. Screening of the rodent feces, collected from backyards and some animal facilities (n = 72), demonstrated also absence of the specific positive results. Despite of that the non-specific products of the reaction have been detected in 2 samples. They were re-tested using classical microbiological testing and additional PCR protocol. Both tests also demonstrated the negative results.
Conclusions: The screening of the environmental samples (soil, dust), feedstuff, rodent feces, and tick collections demonstrated the absence of the causative agent of tularemia in these specimens. Despite of that the necessarily of additional surveillance is obviously, including screening of water samples.

S9-21

The influence of metal ions on the growth of *Francisella subsp. novicida* in water

Tea Jelaca, Andrea Majstorovic, Martina Dajak, Mateja Ozanic, Valentina Marecic, Diana Jurcic-Momcilovic and Marina Santic

Department of Microbiology and Parasitology, Medical Faculty, University of Rijeka, Rijeka, Croatia

Francisella tularensis is a gram-negative facultative intracellular bacterium that resists harsh environment. It has been shown that bacterium is able to survive in water and mud for more than a year. Several epidemics of tularemia have been connected through transmission by water including the recent one in Kosovo and Turkey. Our previous results showed that *F. novicida* survives in natural spring water („Vela Fontana“ from the island of Krk) which contained high concentration of manganese ions. The aim of this study was to determine the effects of different concentrations of Fe, Zn and Mn ions (0.1 μM; 0.8 μM; 0.1 mM; 0.5 mM and 1.0 mM) on the growth of *F. novicida* in ultra pure water by CFU. In addition, the concentrations of metal ions in water were determined by atomic spectrophotometer. The results from this study show that the best growth of *F. novicida* was achieved in water supplemented with Mn ions. The growth rate of bacterium was not dependent on the Mn ion concentrations. In contrast, the concentrations of 0.5 mM and 1.0 mM of Zn ions decreased the growth of *F. novicida* in water while the the concentration of 0.1 μM and 0.8 μM of Zn ions increased the growth of bacterium over time. Similar, the highest concentration (0.5 mM and 1.0 mM) of Fe ions in water decreased the growth of bacteria. However, the concentrations of 0.5 mM and 1.0 mM of Fe did not influence the growth of *Francisella* in water. To conclude, the growth of *F. novicida* is enhanced when the water contains the Mn ions.

Key words: *F. novicida, ions, water*

S9-22

Spatial distribution of *Francisella tularensis* in selected districts of Punjab province, Pakistan

Javed Muhammad[1,5], Masood Rabbani[1], Muhammad Zubair Shabbir[1], Khushi Muhammad[2], Muhammad Wasim[3], Malik Zia Ul Hassnain[1], Haroon Rasheed Chaudhry[1], Muhammad Tasleem Ghori[4], Muhammad haisem[4], Muhammad Asad Ali[2], Tariq Jamil, Girish S Kirimanjeswara[6], Bhushan M Jayarao[5]

[1] University Diagnostic Lab, University of Veterinary and Animal Sciences, Lahore 54600, Pakistan; [2] Department of Microbiology, University of Veterinary and Animal Sciences, Lahore 54600, Pakistan; [3] Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore 54600, Pakistan; [4] Department of Clinical Medicine & Surgery, University of Veterinary and Animal Sciences, Lahore 54600, Pakistan; [5] Animal Diagnostic Lab, Penn State College of Agriculture Science, PennState University, University Park, PA 16802, USA; [6] Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, Pennsylvania, United States of America. Corresponding author's email: javedM81@hotmail.com

Tularemia is a zoonotic disease caused by *Francisella tularensis (Ft)*. This organism can infect a wide range of hosts including invertebrates, birds, and mammals. Humans can become infected when they come in contact with infected animals or contaminated environments such as soil or aerosols or through arthropod vectors. A study was conducted to determine the geo-spatial distribution of *Ft* in soil samples collected from Punjab province of Pakistan. A total of 2,270 soil samples representing 454 villages of eight districts of the Punjab province were examined using real time PCR-based assay. It was observed that 74 (3.25%) of 2, 270 samples were found positive for *Ft* and were from varied locations in Punjab. Interestingly, samples collected from Chakwal district (5.26%) showed higher prevalence while soil samples from Sheikhupura district were negative. Risk factors for *Ft* including environmental temperature and humidity, distance from main road and canal, animal markets, soil cover, animal density, and animal interaction were not associated with presence of *Ft* in the soil samples. Whereas, wind speed (3.451, 1.709-6.968)) and humidity (2.011, 1.234-3.276) was associated with higher frequency of detection of *Ft*, while with increase in the number of human dwellings (0.000; 0.2122-0.7413) the likelihood of detecting *Ft* was significantly lower. These data suggest that *Ft* could be detected in the soil samples of Punjab province by RT-PCR. The observations made in this study can be used to develop hypotheses for a more rigorous analytic epidemiologic study to understand the role of biologic reservoirs of *Ft* and the occurrence of *Ft* in soil samples.

S9-23

Protracted course of human tularemia

Roland Grunow[1], Insa Joost[2], Annerose Serr[3], Cansel Yildiz[4], Philipp Kurz[5], Silke Klee[1], Klaus Heuner[1], Daniela Jacob[1]

[1] Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany; [2] Center for Infectious Diseases and Travel Medicine, Department of Medicine; [3] Center for Microbiology and Hygiene; [4] Department of Oto-Rhino-Laryngology; [5] Center for Pathology, University Hospital of Freiburg, Germany

Francisella tularensis ssp. *holarctica* (F.t.h.) causes usually a relative mild form of tularemia in humans. The mean incubation time is 3-5 days with a range of 1-21 days. In case of suppurative complications like pneumonia and meningitis a prolonged time of convalescence might be required. However, we saw in the past several tularemia patients without serious complications but a prolonged time of recovery or even a re-occurrence of the disease after adequate antibiotic treatment. As an example for a protracted clinical course, we report here a recent German case. The patient, a 22 years old female, is living in an area where previously human cases of tularemia have been seen. The clinical history indicates a tick bite on the leg in June 2014. No further clinical signs occurred. In August 2014, the patient visited Turkey without any hinds for an exposure risk. Mid October, the patient became ill with fever, she also noted a vesiculo-papular rash. After treatment with ciprofloxacin over 5 days due to a simultaneous urinary tract infection, the patient recovered and was well till early December, when she experienced a unilateral cervical lymph node enlargement without other clinical symptoms. The lymph node was mildy tender on palpation and enlarged to the size of a chicken egg. No fistulation or suppuration occurred. In middle of January 2015, the patient was hospitalized for further investigation. A biopsy of the enlarged lymph node revealed an abscess forming granulomatous inflammation with giant cells. A tissue sample was tested positive by 16S-rPCR for F.t., the markers tul4 and fopA confirmed F.t.. The RD1-PCR identified F.t.h.. F.t. bacteria could not been grown. The patient was empirically treated with cefuroxime and metronidazole. She remained sub-febrile (37.3°C). After the diagnosis of tularemia the treatment was switched to oral doxycycline but the temperature did not decline. A further treatment with ciprofloxacin over 14 d cured the sub-febrile temperature. In February 2015, the patient still showed unilateral enlarged lymph nodes and thickening of the cervical muscles, both painful on palpation. The site of the lymph node extirpation showed signs of delayed healing with secretion of a clear fluid (negative for F.t.). The vesicular-papulo exanthema in the cervical region and in the face was still visible. The serology at this time revealed by ELISA and Western blot an anti-Francisella titer of >100,000. Thus F.t.h. can cause long lasting clinical symptoms. The pathologic mechanisms are still unclear.

S9-24

Improved real-time PCR detection of *Francisella tularensis* in challenging waters

Elin Nilsson[1], Linda Jansson[2], Anna Macellaro, Peter Rådström[2], Johanna Thelaus[1], Johannes Hedman[2,3] and Laila Noppa[1]

[1] Division of CBRN Defence and Security, Swedish Defence Research Agency (FOI), SE-901 82 Umeå, Sweden; [2] Applied Microbiology, Department of Chemistry, Lund University, P.O. Box 124,SE-221 00 Lund, Sweden; [3] Section of Biology, National Forensic Centre, SE-581 94 Linköping, Sweden

Detection and quantification of *Francisella* in natural waters can be challenging due to high levels of humic substances and other PCR inhibitors. Here, a study with a pre-PCR processing approach was carried out to find a DNA polymerase-buffer system that tolerates these impurities and provides a robust detection system for the bacteria. Highly inhibitory water and sediment samples were used to evaluate 23 different DNA polymerases for tolerance against PCR inhibition. A standardised real-time PCR assay targeting the *F. tularensis* 17-kD lipoprotein *tul4* gene was used for the screening. The four most promising systems were further analysed using live *F. tularensis* LVS of different concentrations to a range of various waters samples. The samples were surface and sediment lake waters as well as sewage waters and pure waters mixed with animal fecal samples. DNA was prepared using a set of different commercially available extraction kits. Different PCR facilitators were also tested, and BSA gave the best improvement. More robust and sensitive PCR methods will help to improve the possibility to study the natural life-cycle of *F. tularensis*. This is of particular importance since its persistence in nature between outbreaks is not fully understood.

S9-25

First case of *Francisella tularensis* subsp. *mediasiatica* isolation in Altai, south part of Siberia

A. N. Mokrievich[1], V. S. Timofeev[1], T. Yu. Kudryavtseva[1], G. I. Ulanova[2], S. B. Karbysheva[2], R. I. Mironova, G. M. Vakhrameeva[1], T. I. Gubareva[2], V. M. Pavlov[1], I. A. Dyatlov[1]

[1] State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow region, Russian Federation; [2] Center for Hygiene and Epidemiology, Barnaul, Altai Region, Russian Federation

Francisella tularensis is causative agent of tularemia. Degree of virulence of this pathogenic agent is depended on microbe subspecies. There are multiple *F. tularensis* foci in the Russian Federation and neighboring states. It was believed for many years that in Russia circulates only tularemia microbe subsp. *holarctica*. *F. tularensis* subsp. *mediasiatica* was not detected in this area (literature data). However, climate change and probable ability of *F. tularensis* subsp. *mediasiatica* to drift from Central Asian territory in direction of southern regions of Siberia do urgent task of subspecies analysis of *F. tularensis* isolated in this region. *F. tularensis* is characterized by high conservative phenotype (antigenic and biological uniformity, low biochemical activity, etc.), so molecular genetic approaches are used to refer of isolates to certain subspecies and genetic cluster. This analysis is very important for detailed epidemiological study of tularemia microbe circulation. PCR with single primer was developed in SRCAMB for *F. tularensis* subspecies differentiation. The proposed primer Chi1f contains *Escherichia coli* chi-site (5`GCTGGTGG 3`). This site is element of “hot spots” for intragenomic recombination in *E. coli*. Primer Chi1f, in addition to chi-sequence, includes also flanking nucleotides: 5`CTAGG-GCTGGTGG-G 3`. Amplicons obtained by the PCR are typical for *F. tularensis* species and subspecies. A lot of tularemia microbe samples were isolated from ticks, small rodents and water in the Altai Territory recently. This strains were virulent for mice of different lines. Four strains of the collection were studied by multilocus variable number tandem repeats analysis (MLVA) for 25 loci. Three strains analysed were subsp. *mediasiatica*, one strain was subsp. *holarctica.* Intraspecific affiliation of isolates was also confirmed by PCR with Chi1f primer. It was first case of *F. tularensis* subsp. *mediasiatica* isolation in the Russian Federation. *F. tularensis* subsp. *holarctica* strain had Ery^S phenotype. New isolated strains were clustered on the base of MLVA data. Subsp. *holarctica* strain isolated on the border of typical for the Siberian foci of tularemia strains area distribution was inserted in Siberian cluster, slightly differing from them by MLVA-profile. Three subsp. *mediasiatica* strains form new cluster differing from strains isolated in Middle Asia. The data odtained indicate on the circulation as *F. tularensis* subsp. *holarctica* so *F. tularensis* subsp. *mediasiatica* in Altai Area of South Siberia.

S9-26

Modeling of tularemia cases within major endemic regions of Sweden 1984 - 2012

A. Desvars[1], X. Liu[1], A. Sjöstedt[1], A. Johansson[1], P. Rydén[2]

[1] Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, 901 85 Umeå, Sweden; [2] Department of Mathematics and Mathematical Statistics, Computational Life Science Cluster (CLiC), Umeå University, 90187 Umeå, Sweden

Background: Tularemia is mainly recorded in the Northern Hemisphere, but endemic regions are poorly defined and it is unclear if the annual variation of tularemia cases is driven by local or global environmental factors.
Methods: We analyzed 29 years (1984–2012) of nationwide surveillance data from Sweden. The major endemic regions were identified using scan statistics. For each region the annual variation of tularemia cases was modeled using a negative binomial model with five explanatory variables: number of tularemia cases the preceding year, mean summer temperature the preceding year, total summer precipitation, relative mosquito abundance, and number of cold winter days with a minimum snow cover. The explanatory variables were obtained using daily hydrological and meteorological data from hundreds of stations, which were interpolated using a geostatistical inverse distance weighting method. In addition, we fitted global models to each of the regions, which were based on aggregated regional data. By comparing local and global models we quantified to what degree the annual variation of tularemia cases was driven by local and global environmental factors. All models were evaluated considering their pseudo R^2 value and Spearman's correlation between the observed and fitted values (r).
Results: The analysis identified seven major endemic regions (Örebro, Karlstad, Västerdalarna, Ockelbo, Ljusdal, Östersund, and Boden) which accounted for 56.4% of the tularemia cases in Sweden, 9.3% of the population, and 14.2% of Sweden's area. Endemic regions' incidences were 3.8–44 cases/100,000 inhabitants/year while the national incidence was 1.3 cases/100,000 inhabitants/year. The case patients in the endemic regions had similar age and gender distributions (median age = 47−59 years, 46−61% males). For all regions the majority of the case patients were observed from the end of July to the beginning of September. There was a positive correlation between the onset dates of the regions and the geographical distance between the regions (r=0.41, p≤0.05). The local models successfully explained the annual variation of tularemia in five of the seven regions (R^2=0.33−0.76, r=0.54−0.87, all p≤0.01).For several regions the global and local models performed similarly.
Conclusion: Identification of high-risk areas for tularemia is an essential step for outbreak modeling and prevention program management. Models based on environmental and historical case data will be useful for understanding the epidemiological patterns of tularemia in Sweden and for predicting future outbreaks. We provided evidence for not only local but also global drivers of tularemia outbreaks in Sweden.

S9-27

Reemergence of tularemia in the Netherlands

Miriam Koene[1], Joke vd Giessen[2], Jolianne Rijks[3], Miriam Maas[2*], Daan Notermans[2], Ewout Fanoy[2, 4], Marc Engelsma[1], Arjan Stroo[5], Peter van Tulden[1], Kitty Maassen[2], Corien Swaan[2], Mauro De Rosa[5], Ton Oomen[2], Frans Reubsaet[2], Andrea Gröne[3], Hendrik Jan Roest[1]

*[1] Department of Bacteriology and TSEs, Central Veterinary Institute (CVI), part of Wageningen UR, Lelystad, the Netherlands ; [2] National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; [3] Dutch Wildlife Health Centre (DWHC), Utrecht University, Utrecht, the Netherlands; [4] Public Health Service region Utrecht, Zeist, the Netherlands; [5] Netherlands Food and Consumer Product Safety Authority, Centre for Monitoring of Vectors (NVWA-CMV), Wageningen, The Netherlands, * e-mail address: miriam.maas@rivm.nl ; Address: National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (CIb), Centre for Zoonoses and Environmental Microbiology (Z&O), Antonie van Leeuwenhoeklaan 9, P.O. Box 1 , 3720 BA Bilthoven, The Netherlands*

No endemic tularemia was reported in the Netherlands between 1953 and 2011. However, since 2011, tularemia has reemerged in the Netherlands. Five indigenous human infections with *Francisella tularensis* subsp *holarctica* have been reported, as well as 13 infections in hares (*Lepus europaeus*). Infections were found across the Netherlands. The first tularemia patient in October 2011 had no history of foreign travel. Most probably, the route of transmission was via insect bites during a boat trip in the North-East of the Netherlands [1]. Earlier that year, in July, a non-targeted monitoring in hares had already been started in collaboration between veterinary and human public health institutes, responding to an increase in cases in neighboring countries. In May 2013, this monitoring detected the first *F. tularensis* subsp *holarctica*-infected hare in the South of the Netherlands [2]. In July 2013 a visitor of a nature reserve, several kilometers from the location of the positive hare, developed ulceroglandular tularemia through insect bites. The 1953 case also originated from this region. No *F. tularensis* was detected in environmental water samples from this area. In January 2014, a man from the South-West of the Netherlands was infected while skinning a hare that had been caught by greyhounds. He developed fever and a painful swelling in the armpit. Specimens taken from the patient as well as haunches of the hare tested positive for *F. tularensis* subsp *holarctica.* In March 2014, two men in the North-East of the Netherlands became feverish after skinning a hare that was found dead. High antibody titers against *Francisella* were detected in sera. Unfortunately, no material of the hare was available for source confirmation. Since May 2014, there have been no further human cases, but the disease has been confirmed in another 11 hares in the Centre, North and East of the Netherlands. Together with the described human and hare cases this indicates widespread occurrence. Risk assessment, communication, source tracing and surveillance of *F. tularensis* is executed in close collaboration between medical, veterinary and wildlife professionals. In order to explain this reemergence of tularemia in the Netherlands and improve our insight in possible routes of transmission, multidisciplinary research is recommended to improve risk assessment and enable effective preventive measures.

S9-28

Epidemic tularemia outbreak in Russia (Khanty-Mansi autonomous okrug) in 2013

Mazepa A. V.[1], Noskov A. K.[1], Kholin A. V.[1], Balakhonov S. V.[1], Kulikalova E. S.[1], Kozlova I. I.[2]

[1] Irkutsk Antiplague Research Institute of Rospotrebnadzor; [2] Center of Hygiene and Epidemiology in Khanty-Mansi Autonomous Okrug-Yugre 78 Trilisser str., Irkutsk, 664002; fax: +7(3952) 220-140, e-mail: adm@chumin.irkutsk.ru, Russia

In Russia tularemia is registered since 1926 everywhere. Khanty-Mansi Autonomous Okrug (KMAO) is also endemic tularemia territory. Since 1930 to 1950 outbreaks were registered in fact at the whole territory of the Okrug. After 1950 local outbreaks (in 1983 and 2007) and single tularemia cases (2011) occurred. Monitoring of the epizootic condition in the natural tularemia foci showed that in 2009-2011 level of seropositive small mammals was low, but since 2012 increase to 50 % of positively reactive animals (generally red vole - *Clethrionomys rutilus*, common shrew - *Sorex*) was observed. To summer 2013 antibodies to the tularemia causative agent were detected already in 84 % of small mammals. Presence of the active foci near to Khanty-Mansiysk town, increase of the rodents' number, the favorable weather conditions promoting more frequent gonotropical cycles in midges and its number increase, resulted in complication of the epidemiological tularemia condition. In summer 2013 a transmissible tularemia outbreak with prevalence of ulcerobubonic form occurred among inhabitants of Khanty-Mansiysk town and Khanty-Mansi area. In total 1005 cases (63, 37 per 100 000 population) were registered. Eight *Francisella tularensis* subsp. *holarctica* cultures were isolated from patients, four strains - from small mammals. Specific antibodies to the agent were found in blood of small mammals and its antigen - in blood-sucking dipterans. Complex of anti-epidemic measures was performed in the focus: vaccination, implementation of disinfestation, disinsection treatments in natural biotops that resulted in sharp decrease of seropositive samples from animals (15,6 % to the termination of the epidemic outbreak) and interruption of tularemia cases in Khanty-Mansiysk town and Khanty-Mansi region. Next year the in natural KMAO foci seropositive small mammals were revealed in the spring and in the beginning of summer 2014 (82 % and 44 % seropositive animals, respectively) with its further decrease to autumn 2014 (16-19 %). Thus, results of epizootic monitoring in active natural tularemia foci serve as an important signal for timely realization of the preventive measures. Sufficient actions for elimination of epidemic tularemia complications were performed in Khanty-Mansi region in 2013 and demonstrated its efficiency. The estimation of the natural tularemia foci activity revealed a wide epizooty distribution in wild animals in the region that required constant epizootological monitoring conduct for definition of intensity and epizooty distribution limits. Implementation of a complex preventive measures, effective laboratory diagnostics and health service alertness will permit to prevent distribution of human tularemia cases.

S9-29

Genome based analysis of the Swiss *Francisella tularensis holarctica* population

Matthias Wittwer, Christian Beuret, Nadia Schürch

SPIEZ LABORATORY, Federal Office for Civil Protection FOCP, 3700 Spiez, Switzerland

Similar to other European countries there is a growing tendency of tularemia cases in Switzerland. Considering the period 1987-2012 there were on an average 7 reported human cases annually with a subsequent rise to 30-40 cases per year. As national reference center for tularemia an important task is the description of the epidemiological situation in Switzerland. Since ticks are supposed to play an important role as primary vector and/or reservoir for *F. tularensis* and other disease agents our laboratory began in 2009 to collect *Ixodes ricinus* ticks from all over Switzerland. Well over 100'000 ticks have been screened by PCR since and only 0.01% proved to be positive for *F. tularensis holarctica*. On this basis it was possible to define six regions where there is an increased prevalence and which co-localized with reported human infections. Moreover the successful cultivation of *Francisella tularensis holarctica* from tick lysates has confirmed the role of ticks as vectors and enabled phylogenetic typing with a whole genome sequencing approach. In order to elucidate the epidemiological interrelation the genomes of 20 *Francisella tularensis holarctica* isolates originating from patients and ticks were deep sequenced (about 1000 fold coverage) for robust SNP identification. In accordance with recent studies in the field the analysis based on single nucleotide polymorphism (SNP) markers shows that all isolates can be assigned to the two major phylogenetic groups B.FTNF002-00 (dominant in France, Italy and Spain) and B.13 (dominant in Scandinavia, Germany and East-European countries) which are co-circulating in Switzerland although the former group is more prevalent. The phylogenetic tree shows close relationship of the genomes and confirms their clonal origin. Regional grouping of isolates can be perceived at a lower phylogeny level. The occurrence of ticks and human isolates within the same subgroup indicates transfer of the pathogen from tick to human. When mapped against the FTNF002-00 strain the corresponding Swiss isolates show about 100 variants and some of them seem to be suitable for micro-geographic phylogeny. A more in-depth analysis and the sequencing of another 24 isolates from wild animals, humans and ticks are under way. With this holistic approach we expect to gain a more detailed understanding about the natural infection cycle and the epidemiological potential of *Francisella tularensis holarctica* in Switzerland.

S9-30

Presence of *Francisella tularensis* in mosquitoes of central Poland

K. Formińska[1], A. Masny[2], E.Gołąb[2], A.Zasada[1]

[1] Department of Bacteriology, National Institute of Public Health- National Institute of Hygiene, Warsaw, Poland; [2] Department of Medical Parasitology, National Institute of Public Health- National Institute of Hygiene, Warsaw, Poland; kforminska@pzh.gov.pl

Tularemia is a zoonotic disease caused by *Francisella tularensis* a Gram- negative and intercellular coccobacillus. *F. tularensis* is transmitted to humans either by a number of different routes including direct contact with infected wild animals, aerogenic exposure, contaminated water or food, inhalation of aerosols, or by vectors such as ticks, mosquitoes and deer flies. Reservoir of hosts can include lagomorphs, rodents, galliform birds and deer. Tularemia is endemic in most European countries. The number of confirmed cases is usually less than 100 cases per year. In Poland 52 cases were registered in the period of seventeen years, of which 6 and 8 cases were reported in 2012 and 2013, respectively. Majority of the reported cases of tularemia in Poland had septic or typhoidal forms, developing after an arthropod bite. In the reports from Scandinavia the transmission of tularemia to humans has been attributed to mosquitoes bites. In Poland no research has been conducted, to confirm the presence of *F. tularensis* in arthropods or insects. The aim of our study was to verify the mosquitoes as potential vectors of tularemia. For this purpose 2180 mosquitoes were collected, during summer 2011 and 2012, in a residential area in central Poland. After DNA extraction samples were examined by PCR assay for detection of *tul4* gene of *Francisella tularensis.* In the examined samples no DNA of *F. tularensis* was detected which indicates that the rate of infection of mosquitoes could be lower then 1/2000. If the risk of transmission of *Francisella tularensis* to humans via mosquitoes bites existed in this region of Poland it would be very low.

S9-31

Yersinia pestis and *Francisella tularensis* in vectors and rodents in northern Azerbaijan

Huseyn Hajiyev, Bakhtiyar Aydinov, Azad Jafarov

Khachmaz Anti-Plague Division, Khachmaz, Azerbaijan, 3 Heydar Aliyev, Khachmaz AZ2700 Azerbaijan

The especially dangerous pathogens, *Yersinia pestis* (causative agent of plague) and *Francisella tularensis* (causative agent of tularemia) are known causes of zoonotic disease. Currently, the distribution of plague and tularemia in Azerbaijan is unknown, as little funding has been allocated in recent years for surveillance activities. This study will examine the ecological and epidemiological characteristics of *Y. pestis* and *F. tularensis* in the Khachmaz and Gusar rayons in northern Azerbaijan. These rayons are adjacent to the Dagestan Republic, which has had recent cases of plague and tularemia and has a similar geography. Arthropods infesting wild rodent burrows and collected off trapped rodents will be collected over 6 months in 13 villages, with 9 days spent at each village. Blood samples will also be collected from the rodents. Rodents will be identified in the field, while arthropods will be identified at the Khachmaz Anti-Plague Division (APD) BSL-2 laboratory. PCR targeting two genes for each pathogen will be used to detect *Y. pestis* and *F. tularensis* in both the vectors and the rodent blood to determine the prevalence and distribution of these agents in the arthropods and their associated hosts. The data will be compared with the results from other studies conducted on the Azerbaijan-Dagestan border. This project will provide valuable information on the prevalence of plague and tularemia in rodent populations in Northern Azerbaijan, and will help to guide future surveillance efforts.

S9-32

Francisella species in ticks and animals, Iberian peninsula

Lopes de Carvalho I[1], Toledo A[2,3,4], Carvalho CL[5,6], Barandika JF[7], Respicio-Kingry LB[8], Garcia-Amil C[2], García-Pérez AL[7], Olmeda AS[3], Zé-Zé L[5], Petersen JM[8], Anda P[2], Núncio MS[1,5], Escudero R[2]

[1] Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal; [2] Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; [3] Universidad Complutense de Madrid, Madrid, Spain; [4] Current affiliate: Stony Brook University, Stony Brook, New York, USA; [5] Instituto Nacional de Saúde Dr. Ricardo Jorge, Águas de Moura, Portuga; [6] University of Évora, Évora, Portugal; [7] NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Biscay, Spain; [8] Centers for Disease Control and Prevention, Fort Collins, Colorado, United States of America

The most widely recognized species within the *Francisella* genus is *F. tularensis*, the causative agent of tularemia, a disease that can be lethal to humans and to a wide variety of animal. *F. hispaniensis* is a more recently described member of the genus, initially described after isolation from a patient with severe illness in Spain. Currently, its distribution in nature is unknown. In Portugal, *F. tularensis* subsp. *holarctica* was first reported in *Dermacentor reticulatus* and in humans in 2007 whereas FLEs were first detected in *D. reticulatus* in 2010. In Spain, sporadic human cases of tularemia associated with tick bite have been reported, since the first reported outbreak in 1997. FLEs have been regularly identified in a variety of tick species in Spain. Here we report the presence of *F. tularensis* subsp. *holarctica* in lagomorphs, small mammals and different tick species from the Iberian Peninsula. We also report for the first time DNA detection of *F. hispaniensis*-like organisms and *Francisella*-like endosymbionts in small mammals. Ticks were collected from vegetation as well as from wild and domestic animals. Rodents from the same areas were trapped, and tissues were also collected from European brown hares and European rabbits. DNA was extracted and tested using a real-time multitarget TaqMan PCR, using *tul4* and IS*Ftu2* assays, and a conventional PCR targeting *lpn*A. For additional characterization, succinate dehydrogenase A (*sdhA*) and VNTR Ft-M19 were also amplified. Amplicons obtained by *lpnA, sdhA* and Ft- M19 PCR were purified and sequenced. A neighbor-joining tree of DNA sequence alignment was built. The presence of *Francisella* species in 2134 ticks, 93 lagomorphs and 280 small mammals from the Iberian Peninsula was studied. Overall, 19 ticks and 6 lagomorphs were positive for *F. tularensis* subsp. *holarctica*, suggesting, as described for other regions, that lagomorphs may have an important role in the maintenance of *F. tularensis* in nature. Additionally, 353 ticks and 3 small mammals were PCR positive for *Francisella*-like endosymbionts and one small mammal was also positive for *F. hispaniensis*-like DNA sequences. A variety of *Francisella*-like endosymbiont sequence types were detected: ticks were associated with 5 *lpnA* sequence types, with only one type identified per tick, in contrast to 2 *lpnA* sequence types detected in a single wood mouse (*Apodemus sylvaticus)*. To our knowledge, this is the first report of *Francisella*-like endosymbionts in free-living small mammals as well as the first detection of *F. hispaniensis*-like sequences in a natural setting.

S9-33

A glandular tularemia case report from an unusual geographic location in Georgia

Tinatin Kuchuloria[1,2], Marina Endeladze[3], Nazibrola Chitadze[1], Tamar Chikviladze[1], Mariam Broladze[1], Natalia Abazashvili[1], Tamar Giorgadze[1], Paata Imnadze[1], Tengiz Tsertsvadze[3], Nino Akhvlediani[1,2], Tamar Akhvlediani[1,2], Louise-Anne McNutt[4], Robert G. Rivard[5], Gvantsa Chanturia[1]

[1] National Center for Disease Control and Public Health, Tbilisi, Georgia; [2] US Army Medical Research Unit Georgia, Tbilisi, Georgia; [3] Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia; [4] University at Albany, State University of New York, NY, USA; [5] US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA

Tularemia was first reported in the country of Georgia in 1946. Since that time, sporadic cases and water-borne outbreaks have been reported in rural areas from natural foci. In 2013, as part of the Cooperative Biologic Engagement Program funded by the US Defense Threat Reduction Agency a project studying tularemia in humans, vectors and reservoirs in Georgia was launched. To improve tularemia case detection, active syndromic surveillance began in January 2014 at eight hospitals in Georgia, including healthcare facilities in the natural foci and reference centers in the capital. Enrolled patients were >18 years of age, had fever >38°C, undefined diagnosis, and at least one tularemia syndrome. Blood cultures were collected to isolate *Francisella tularensis*. Antibodies against *F. tularensis* were detected via serologic tests (IgM and IgG ELISA, MAT). As part of the study antibodies against *Brucella spp*. were detected via IgM and IgG ELISA, and antibodies against *Coxiella burnetii* were detected via Phase II IgM and IgG ELISA, as well as Phase I IgG ELISA. To date, eleven patients have been enrolled. Only one was confirmed as a glandular tularemia case. A 50-year-old female, a resident of Tbilisi, was admitted to the infectious diseases reference hospital in early December 2014, (two months after disease onset) with a high fever (>38^{0}C) and cervical lymphadenopathy. The patient lives in a private house with a yard and has exposure to several cats. No travel was reported for the month before symptom onset. Paired sera *F. tularensis* IgM and IgG ELISAs were positive. The MAT titers for acute and convalescent sera were 1:8129 and 1:4096, respectively. Blood cultures and ELISAs (*Brucella spp.* and *C. burnetii*) were negative. As part of the routine medical care bartonellosis, toxoplasmosis, CMV and EBV infections, TB, and HIV-infection were excluded. The patient refused a diagnostic bubo biopsy. The patient was administered IV ciprofloxacin (400 mg BID) and PO doxycycline (100 mg BID) but this failed to halt progression. After initiation of IV gentamycin (5 mg/kg), defervescence was observed, and bubo tenderness and redness resolved. Three weeks later, upon discharge, the cervical bubo was still present. Our surveillance results suggest the clinical manifestations of tularemia among patients at the selected clinical sites during the surveillance period were quite limited. Only one case was confirmed. Although exposure to cats and other carnivores is not a major transmission route for humans, during case investigations, consideration should be given to less common exposures.

S9-34

Field planning based on ecological Niche models of *Francisella tularensis* isolated from *Microtus arvalis* and *Dermacentor marginatus* in Georgia

I. Burjanadze[1], N. Tsertsvadze[1], M. Shavishvili[1], I. Shalutashvili[1], A. Dzneladze[1], A. Navdarashvili[1], G. Chanturia[1], P. Imnadze[1], I. Kracalik[2,3]; J. K Blackburn[2,3]

[1] National Center for Disease Control and Public Health (NCDC) Tbilisi, Georgia; [2] Spatial Epidemiology and Ecology Research Lab, Department of Geography, University of Florida, Gainesville, FL, USA; [3] Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA.

Introduction: Tularemia was first described in Georgia over 70 years ago. Since then, it has sporadically re-emerged in several regions of the country as focused human cases and as large outbreaks. Recently, the US Defense Threat Reduction Agency (DTRA) funded project "Epidemiology and Ecology of Tularemia in Georgia" (GG-19) was initiated to study *Francisella tularensis*, the causative agent of tularemia. One of the aims of this project was to create ecological niche models of *F. tularensis* using field collection data in several hypothesized reservoirs: *Microtus arvalis* (common vole) and *Dermacentor marginatus* (ornate sheep tick). These predictive models can be used to help derive future sampling strategies and field locations.

Methods: Historical records and archival data (1956-2011) of tularemia outbreaks for 465 strains were retrieved from records housed at NCDC. In addition, contemporary data from environmental field collections during 2013-2014 were used to build a geographic information system (GIS) database. In total, we used data from four separate regions of Georgia. To model the potential geographic distribution of *F. tularensis* in the studied vole and tick species, we used the Genetic Algorithm for Rule-set Prediction (GARP). This is the only modeling approach (drives pseudo-absence) that is used in conjunction with environmental/climatic variables to identify correlative patterns on the landscape. Model predictions of the hypothesized reservoirs of tularemia in Georgia were then used for planning field sample collection and seroprevalence study activities.

Results: Model experiments showed good discrimination between presence and pseudo absence. The area under the curve (AUC) for both models was > 0.80. In general, models showed high agreement (7 models or better) in two regions of Georgia, excluding most of the western portion of the country. As a result, areas were targeted in southern and eastern Georgia where field collection and seroprevalence study teams could conduct surveillance.

Conclusion: Our findings identified suitable niches for *F. tularensis* and its reservoirs. Our results demonstrate the utility of predictive modeling in better understanding the ecology and geographic distribution of *F. tularensis*. These new results not only provide information about the ecology of tularemia, but also aid in the allocation of limited resources for continued surveillance and monitoring activities. To that end, our sample collection activities and seroprevalence study were continued in the areas with the highest model agreement. These techniques can be used in the prevention and control of other zoonoses in Georgia.

S9-35

Tularemia in children in Kosovo from November 2014 - March 2015

Sadiku I.,Bajrami M., Ramadani H., Mehmeti M., Ponosheci A., Berisha V.

University Clinical Center of Kosovo, Clinic of Infectious Diseases, Kosovo

First cases of Tularemia in Kosovo were confirmed in 2000, in municipality of Gjakova, village Brovina, where were reported 22 cases. During 2000-2002 there was an epidemic with more than 600 cases. In 2014-2015 there were around 300 cases of Tularemia, 115 of them were treated at Clinic for Infectious Disease, 33.9% of cases were children. The aim of this study is to present the characteristics of tularemia in pediatric ages in Kosovo during November 2014-March 2015. Data were analyzed for clinical and epidemiological characteristics and treatment of the disease.
Methods: We have analyzed 39 patients that were treated in Clinic for Infectious Disease in Pristina. In this study we have analyzed data from their medical records which include anamnesis, clinical examinations, laboratory tests and treatment.
Results: Cases with Tularemia that were treated in our Clinic were from the same 9 municipalities that were involved in epidemic in 2010 in Kosovo. Mean age of patient was 10.2 y.o., 51.2% patients were under 10 y.o. of age. Social-epidemiological conditions were very important factor in appearance of the disease, 53% of patients were water supplied only from wail. All the patients were from rural areas. Clinical Manifestations were: temperature, neck pain, neck and inguinal lymphadenopathy, and tiredness. Glandular form of disease has dominated. From laboratory tests 95% (20) patients had high Erythrocyte Sedimentation. Rate Agglutination test was positive in all cases. All cases were treated with antibiotics, 100%. 15% of cases have repeated treatment with antibiotic therapy. Incision and drainage of lymph node as additional therapy was applied in (10) patients, 25.6%.
Conclusion: Tularemia continues to be one of the diseases, which in late last decade poses health problem for our country. In Kosovo continues to dominate the glandular form of tularemia. Treatment of tularemia with gentamycin was effective. Incision and drainage of the inflamed gland has shown to be good method to accelerate improvement of the patient.

S9-36

Francisella tularensis clades B.FTN002-00 and B.13 are associated with distinct pathology in the European brown hare (*Lepus europaeus*)

Francesco C. Origgi[1,2], Paola Pilo[1]

[1] Institute for Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Center for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Recent studies suggest that distinct clades of *F. tularensis* subsp. *tularensis* with specific geographical distribution are associated with different outcomes in humans, virulence in mice, ecological niches, and host-range species. However, these aspects are still poorly characterized as concerns the subpopulations belonging to the subspecies *holarctica*. In Europe, two major clades of *F. tularensis* subsp. *holarctica* are currently circulating: the B.FTNF002-00 in the west and the B.13 in the rest of the continent. This study investigated the pathology and microbiology of Swiss free-ranging European brown hares (*Lepus europaeus*) naturally infected with *F. tularensis*. Carcasses of hares were collected between February 2012 and May 2014. Gross and histopathological examinations were carried out on all of them and multiple organs were tested by direct PCR and by culture. Moreover, immunohistochemistry was performed. Isolates of *F. tularensis* were further phylogenetically typed and phenotypic and genotypic antibiotic resistance profiles were characterized. Twenty-eight out of 53 hares were positive for *F. tularensis*. Isolates were characterized as *F. tularensis* subsp. *holarctica* belonging to the clades B.FTNF002-00 (n=26) and B.13 (n=2) (more specifically the subpopulations B.33/34 and B.34/35). Isolates belonging to the group B.13 were previously shown to be erythromycin resistant in association with mutations in the *rrl* and *rplD* genes, while isolates from the clade B.FTNF002-00 were sensitive to erythromycin. Pathological investigation revealed that spleen, liver, lung, lymph nodes and trachea were more frequently involved but other organs, including adrenal glands, were also affected to variable extent. The features of the lesions were similar to those described in humans and in multiple animal species. However, the distribution and severity of the lesions was different from that described in hares infected with the clade B.13. This study showed that the clade B.13, displaying resistance to macrolides, is more widespread in Western Europe than previously described. The pathology findings in the examined hares are consistent with B.FTNF002-00-associated lesions being different than those previously described in B.13 infected European brown hares.

S9-37

Microbial genomic analysis indicate environmental persistence of tularemia in Bulgaria over three decades of epidemiological silence

Kerstin Myrtennäs[1*], Krustyu Marinov[2*], Anders Johansson[3], Edvin Karlsson[1], Mona Byström[1], Mats Forsman[1]

**These authors contributed equally to this work.*

[1] Swedish Defence Research Agency, Cementvägen 20, S-901 82 Umeå, Sweden. [2] Military Medical Academy, Sveti Georgi Sofiyski 3, 1606 Sofia, Bulgaria. [3] Umeå University, S- 901 85, Umeå, Sweden; Kerstin Myrtennäs, Swedish Defence Research Agency, Division of CBRN Defence and Security, SE-901 82 Umeå, Sweden; e-mail: kerstin.myrtennas@foi.se.

In 1960s a large tularemia outbreak occurred in the Srebarna lake reserve in Bulgaria followed by 30 years with no reported tularemia until a new outbreak occurred in the 1990s. Previous studies of tularemia in other countries have shown that *Francisella tularensis* could persist in restricted geographical areas for many years. Therefore, to explore whether strains isolated in the 1960s and 1990s outbreaks were related, we analyzed a sequence variation dataset including ten new *F. tularensis* subspecies *holarctica* genome sequences from Bulgaria, and placed these strains into the current global taxonomic framework of *F. tularensis* based on canonical SNPs (canSNPs). The genetic analysis showed that *F. tularensis* isolates within a previously described genetic clade named B.34 and in a new clade, both within the basal clade B.12, were present in both the 1960s and 1990s outbreaks, indicating local persistence of tularemia in Bulgaria. In contrast, a single strain B1 within the basal clade B.4 and isolated in 1999 showed closest kinship with strains from other countries indicating a more recent appearance of this clade in Bulgaria. Another single strain named 19c within the basal clade B.12 and isolated in Srebarna lake reserve already in 1961 was only at a distance of two SNPs from a strain named 94 and isolated in the former Soviet Union in 1956, supporting an earlier introduction of this clade into Bulgaria. In future investigations, four new canSNP assays developed in this work can be used to identify the specific genetic clades where isolates from Bulgaria are members. In conclusion, this study provides information on the genetic diversity of *F. tularensis* in Bulgaria.

S9-38

Performance of serological tests for diagnosis tularemia in Peja region, January - April 2015

Vanina Kryeziu-Gafuri, Fatmire Kollçaku, Shqipe Krasniqi-Kola

National Institute of Public Health of Kosova- Peja branch, Pec, Kosova

Intoduction: The bacterium (*Francisella tularensis*) is highly virulent for humans and a range of animals such as rodents, hares and rabbits. It may cause epidemics and epizootics. *F. tularensis* is transmitted to humans by arthropod bites, by direct contact with infected animals, infectious animal tissues or fluids, by ingestion of contaminated water or food, or by inhalation of infective aerosols. There is no human-to-human transmission. Purpose this study is to present clinical and laboratory characteristics, as well as the method and effect of treating tularemia.
Methods: Most common serological tests for tularemia disease are MAT (microagglutination assay test), ELISA classic *Francisella tularensis* IgG/IgM and Western Blot (WB). In this study the diagnosis of tularemia was established using a standard micro-agglutination assay and ELISA IgG/IgM in total 41 sera tested during period January-April 2015 in NIPH.
Results: In total 41 sera from suspected cases were tested with MAT, from which 19 (46.3%) were positive in tularemia. 19 sera were then tested by ELISA and the resulting positive, from them 12 (63.1%) IgM positive and 7(36.9%) IgG positive. Results show domination of anginouse form of disease with one-sided angina, febrility , cervical adenopathy.and oculoglandular tularemia.
Conclusion: The diagnostic value of the commercial assays was proven. The sensitivity of the MAT (microagglutination assay test) and ELISA classic IgG/IgM were acceptable and very specific and had a very good positive predictive value.

S9-39

Tularaemia in Peja region-Republic of Kosova, 1999 to 2014

Fatmire Kollçaku,Shqipe Krasniqi-Kola,Vanina Gafuri,Besa Nila

National Institute of Public Health of Kosova-Peja branch, Pec, Kosova

Introduction:Tularaemia is a bacterial zoonotic disease of the northern hemisphere,caused by the bacterium *Francisella tularensis.* There is no human-to-human transmission.The disease is a more serious public health problem in Balkan than other Europe countries.
The purpose of the paper: This study aims to provide a follow-up on the incidence of tularaemia in Peja Region,from the first outbreak in 1999 until 2014.
Material and methods: A national surveillance system has been implemented in Republic of Kosova since 2000 to monitor a number of diseases, including tularaemia.For this study we used reporting forms,based on syndromic approaches and clinical diagnoses.They were obtained by local physicians from suspected cases and sent for analysis to the national Institute of Public Health.Therefore, laboratory conformation by detecting specific antibodies or the pathogen itself is required for the final diagnosis of tularaemia.Cases laboratory confirmed by ELISA and western blot. To research this issue we using descriptive and analitical methods, based on retrospective and prospective research of this desease.
Results: After the tularaemia outbreak in 1999–00, a second outbreak occurred from November 2001 to June 2002, which was investigated by a Kosovar/German team.During this period, 278 serum samples from suspected cases in Peja Region, were tested; 125 cases laboratory confirmed, with average incidence 55,5 / 100,000 inhabitants. After the first and second outbreak, the surveillance system revealed the presence of cases every year. From 2001 to 2014,0-19 cases were registered per year. In relation to municipalities, 91 cases 72,8% were registered in Klina Municipality. Housewives and students were the most affected occupational groups, representing 39% (n=49) and 37%(n=47) of cases, respectively. This is also reflected in the sex distribution of all cases during this period: 59% (n=74) of cases were female in first outbreak,and 83% male in second outbreak. Most cases,46% (n=58) were in the age group 20–40 years. In addition, quite a high proportion, about 38% (n=48) of children and teenagers (aged under 20 years) were infected.The investigation of animal and environmental specimens by the capture ELISA, which is highly specific for *F. tularensis*, showed that the antigen was detected mainly in mouse and hare faeces
Conclusion: As in the first and second outbreak, most of the tularaemia patients in Peja Region, during 2003 to 2014,had the oropharyngeal form with fever and a unilateral cervical lymph node enlargement as the main symptoms.In fact, the data for 2003 to 2014 indicate a continuous activity of tularaemia after the initial outbreaks. Given the situation, ingestion of contaminated food and water arising from the poor hygiene conditions seem to be the most likely risk factors for the infection. It was characteristic of both outbreaks that people in affected regions reported an enormous increase in the rodent population In addition, animal control and surveillance, including that of rodents, should be carried out to prevent further outbreaks.Also health education.

S9-40

Outbreak of tularaemia in Peja region - Kosovo, January to April 2015

Shqipe Krasniqi-Kola, Fatmire Kollçaku, Adnora Nurboja, Vanina Gafuri

National Institute of Public Health of Kosova - Peja branch, Pec, Kosova

Introduction: Tularaemia is a zoonotic disease caused by *Francisella tularensis*. First cases of disease have been registered in Kosovo in an outbreak in 1999, including Peja Region. Since then, over the years have been also registered new cases of tularaemia. On 10 February 2015, the Committee for the Prevention of Infectious Diseases at the Kosovo Ministry of Health declared an outbreak of tularaemia in the country. The purpose of this study is to describe the incidence and epidemiological characteristics of tularaemia in Peja Region from January to April 2015.

Material and Methods: Clinical and epidemiological information is obtained from clinical notification forms and supplemented from questionnaires taken from the patients. Laboratory results were available from the laboratory notification form for each case. Specific antibodies were detected in the serum, using a combination of agglutination technique, as well as ELISA for IgG and IgM antibodies. Descriptive method of work is used and the data obtained are analyzed in chronological, demographic, and topographic terms.

Results: During the period January to April 2015 are registered 19 cases of tularaemia in the Peja Region with average incidence 8.4 / 100,000 inhabitants, with significant differences between municipalities (16 cases 84.2% were registered in Klina Municipality); 14 (73.7%) were males; the median age was 30.3 years (range 2-56 years); 18 (94.7%) were from rural areas. Most patients had oropharyngeal or glandular form of tularaemia-89.4% of the cases, followed by oculoglandular (5.3%) and cutaneous form (5.3%). It is characteristic that people in affected region reported an enormous increase in the rodent population, especially in their living and working areas.

Conclusions: Tularaemia has been a notifiable disease in Kosovo since 1999, including Peja Region. From 1999 to 2015 the annual incidence in Peja Region has varied from 0 to 19 cases. Since 2010 Kosovo represented an endemic region for tularaemia. In the present outbreak in Peja Region, the clinical picture of oropharyngeal tularaemia points to ingestion of contaminated food or water, as the most likely route of transmission. An unusually large increase in the rodent population was observed, which can facilitate the spread of zoonotic infectious pathogens among animals and induce an epizootic spread to man. An animal control, including that of rodents should be carried out to prevent further outbreaks. Physicians in the endemic area should be reminded of the need to consider tularaemia in patients with febrile illness and of how to make the diagnosis.

Session 10:

Short Presentations

Session 10: Short Presentations

Chairs: Mats Forsman
Swedish Defence Research Agency, Sweden

Miklos Gyuranecz
Institute for Veterinary Medical Research, CAR-HAS, Hungary

S10-1 Stimulus responsive mesoporous silica nanoparticles provide controlled release of moxifloxacin and enhanced efficacy against pneumonic tularemia in mice
Bai-Yu Lee, Zilu Li, Daniel L. Clemens, Barbara Jane Dillon, Angela Hwang, Jeffrey I. Zink and Marcus A. Horwitz

S10-2 No evidence of transovarial transmission of *Francisella tularensis* by tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*
Massimo Fabbi, Paola Prati, Nadia Vicari, Andrea Manfredini, Luciano Sacchi, Emanuela Clementi, Claudio Bandi, Sara Epis, Marco Genchi

S10-3 Detection of virulence difference between B.13 (“red”) and FTN002-00 (“purple”) *Francisella tularensis* ssp. *holarctica* genotypes
Zsuzsa Kreizinger, Károly Erdélyi, Massimo Fabbi, Miklós Gyuranecz

S10-4 Analysis of *Francisella tularensis* strains isolated from humans and small rodents during year 2013 tularemia outbreak in Khanty-mansiysk sity, West Siberia
V.M. Pavlov, I.I. Kozlova, A.N. Mokrievich, O.D. Shutko, V.S. Timofeev, R.I. Mironova, T.S. Kuznetsova, N.M. Fayzullina, T.Y. Kudryavtseva,T.I. Kombarova, I.A. Dyatlov

S10-5 Gallium potentiates the antibacterial effect of gentamicin against *Francisella tularensis*
Helena Lindgren and Anders Sjöstedt

S10-6 Qualification of cynomolgus macaque model of pulmonary tularemia
Lynda Lanning, Patrick Sanz, Larry Wolfraim, Christopher Houchens, Kristian Omland, Steven Heyse, Robert Johnson, Ed Nuzum, Judith Hewitt, Tina Guina

S10-1

Stimulus responsive mesoporous silica nanoparticles provide controlled release of moxifloxacin and enhanced efficacy against pneumonic tularemia in mice

Bai-Yu Lee[1*], Zilu Li[2*], Daniel L. Clemens[1*], Barbara Jane Dillon[1], Angela Hwang[2], Jeffrey I. Zink[2,3], and Marcus A. Horwitz[1]

**Contributed equally*
[1] Division of Infectious Diseases, Department of Medicine, School of Medicine, University of California–Los Angeles ; [2] Department of Chemistry & Biochemistry, University of California–Los Angeles; [3] California NanoSystems Institute, University of California–Los Angeles, Department of Medicine, CHS 37-121, UCLA School of Medicine, University of California–Los Angeles, 10833 Le Conte Avenue, Los Angeles, California 90095-1688,USA

Effective and rapid treatment of tularemia, especially the inhalational form, is needed to reduce morbidity and mortality of this serious and potentially fatal infectious disease. The etiologic agent of tularemia, *Francisella tularensis*, is a facultative intracellular bacterial pathogen and Tier 1 Select Agent that infects macrophages and multiplies to high numbers in these host cells. Nanotherapeutics are particularly promising for treatment of infectious diseases caused by intracellular pathogens whose primary host cells are macrophages because nanoparticles preferentially target and are avidly internalized by macrophages. We have developed two mesoporous silica nanoparticle (MSN) platforms for delivery and release of antibiotics in response to intracellular cues. The first MSN platform is functionalized with pH-sensitive valves that remain closed at the neutral pH of the blood, but open and release drug after endocytosis into acidified endolysosomes. The second MSN platform has very high drug loading and is equipped with disulfide snap-tops that selectively release drug after endocytosis in response to the intracellular redox potential. These nanoparticles, when loaded with Hoechst fluorescent dye, release their cargo exclusively intracellularly and stain the nuclei of macrophages. We demonstrate the utility of these two types of MSN carriers by comparing the efficacy of MSN-delivered moxifloxacin, a broad spectrum antibiotic, vs. free moxifloxacin in killing *F. tularensis* in infected macrophages *in vitro* and in treating pneumonic tularemia in vivo. PMA-differentiated THP-1 macrophages were infected with *F. tularensis* Live Vaccine Strain and subsequently mock treated or treated with moxifloxacin or with the two different types of MSNs loaded with moxifloxacin. Both types of moxifloxacin-loaded MSNs killed *F. tularensis* in macrophages in a dose-dependent fashion; in this *in vitro* system, the drug-loaded MSNs had the same potency as an equivalent amount of free drug. *In vivo* efficacy of the moxifloxacin-loaded MSNs was evaluated in a mouse model of pneumonic tularemia in which mice were challenged with ~6-fold the LD_{50} of *F. tularensis* LVS by the intranasal route. Both types of moxifloxacin-loaded MSNs prevented weight loss, illness, and death in the *F. tularensis*-challenged mice, markedly reduced the organ burden of

F. tularensis in the lung, liver and spleen, and were significantly more efficacious than an equivalent amount of free drug. This study provides an important proof-of-principle for the potential therapeutic use of two novel MSN drug delivery platforms for the treatment of tularemia and potentially other infectious diseases caused by intracellular pathogens.

S10-2

No evidence of transovarial transmission of *Francisella tularensis* by tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*

Massimo Fabbi[1], Paola Prati[1], Nadia Vicari[1], Andrea Manfredini[1], Luciano Sacchi[2], Emanuela Clementi[2], Claudio Bandi[3], Sara Epis[3], Marco Genchi[1]

[1] National Reference Laboratory for Tularemia, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Pavia, Italy, [2] Department of Biology and Biotechnology (Lazzaro Spallanzani), Università degli Studi di Pavia, Pavia, Italy, [3] Department of Veterinary Science and Public Health (DIVET), Università degli Studi di Milano, Milan, Italy; Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), Pavia Department, Strada Campeggi, 59/61 – 27100 Pavia, Italy. Phone: +39 0382422006, fax: +39 0382 422066

Due to its easy dissemination, multiple routes of infection, high environmental contamination and morbidity and mortality rates, *Francisella tularensis* is considered a potential bioterrorism threat and classified as a category A select agent by the CDC. Tick bites are among the most prevalent modes of transmission, and ticks have been indicated as a possible reservoir, although their reservoir competence has yet to be defined. Tick-borne transmission of *F. tularensis* has been recognized since 1923, and transstadial transmission has been demonstrated in several tick species. Studies on transovarial transmission, conducted between 1920 and 1960, when molecular techniques had not been developed nor *F. tularensis* subspecies had been defined yet, have produced conflicting results. The aim of this study was to evaluate the role of ticks as reservoirs for *Francisella*, assessing the transovarial transmission of fully virulent *F. tularensis* subsp. *holarctica* in experimentally-infected females of *Dermacentor reticulatus* and *Ixodes ricinus*. The experimental design was performed in 6 replicates. A total of 150 *D. reticulatus* and 150 *I. ricinus* unfed questing adult female ticks were used. For each replicate, 2 guinea pigs were used. On Day -3, all animals were infected with 25 female ticks and 35 male ticks and on Day 0, the tick-infested guinea pigs were inoculated subcutaneously with 500 CFU of the bacterium suspended in 0.3 ml of sterile saline solution. After completion of the tick blood feeding, bacterial culture and real-time PCR confirmed the infection by *F. tularensis* subsp. *holarctica* in all animals. All ticks examined during and/or at the end of oviposition were positive by PCR and culture. PCR, culture, transmission electron microscopy and fluorescence *in situ* hybridization showed *F. tularensis* within tick oocytes. However, cultures and bioassays of eggs and larvae were negative; in addition, electron microscopy techniques revealed bacterial degeneration/death in the oocytes. These results suggest that bacterial death might occur in oocytes, preventing the transovarial transmission of *Francisella*. We can speculate that *Francisella* does not have a defined reservoir, but rather various biological niches (e.g. ticks, mosquitoes, rodents, lagomorphs, amoebae) that allow the bacterium to persist in the environment. Our results suggest that ticks are not competent for the vertical transmission of the bacterium and are congruent with this view. However, even in the absence of a transovarial transmission, ticks are able to maintain the infection in the environment during the inter-epizootic period and can be identified as long-term vectors of *F. tularensis*.

S10-3

Detection of virulence difference between B.13 (“RED”) and FTNF002-00 (“PURPLE”) *Francisella tularensis* ssp. *holarctica* genotypes

Zsuzsa Kreizinger[1], Károly Erdélyi[2], Massimo Fabbi[3], Miklós Gyuranecz[1]

[1] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, (m.gyuranecz@gmail.com); [2] Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary; [3] Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna (I.Z.S.L.E.R.), Pavia, Italy

The *Francisella tularensis* ssp. *holarctica* population in Europe is classified into two main genetic subclades. The B.13 subclade predominates in central and eastern Europe while the FTNF002-00 is the only native subclade in the western part of the continent. The European brown hare (*Lepus europaeus*) is considered the main reservoir species of tularemia in central Europe. The B.13 type strains from this region cause subacute/chronic disease in hares with typical granulomatous lesions in the lung, pericardium and kidney. However, European brown hares dying from tularemia caused by the FTNF002-00 strain in western Europe generally show signs of septicaemia with enlarged spleen and liver during necropsy. The aim of this study was to examine whether there is a difference in the virulence of B.13 and FTNF002-00 genotypes *of Francisella tularensis* ssp. *holarctica* strains. Groups of six age-matched (7 weeks) Fisher rats were infected intraperitoneally with 10^0, 10^1 and 10^2 CFU of the B.13 genotype (Hungarian strain) and FTNF002-00 genotype strains (Italian strain). Six rats served as negative controls. Clinical signs and body weights of the rats were checked daily, and after 21 days all the rats that did not succumb to the infection were euthanized. Samples from the dead rats were submitted for serological, pathological and histopathological examinations. No significant difference was detected between the different inoculation doses. However, rats infected with the FTNF002-00 genotype strain developed a more severe disease than those infected with the B.13 genotype isolate. Seven out of the 18 rats inoculated with the FTNF002-00 genotype strain died (3-5 days post inoculation /pi./) while only 3 of the 18 rats infected with the B.13 genotype strain succumbed to the infection (3-4 days pi.). Clinical signs were similar in both groups: decreased appetite, water consumption and weight loss between days 4-13 pi., accumulated porphyrin around the eyes and nose and diarrhea. The deceased rats were seronegative while all surviving animals showed positive reaction in slide agglutination test on day 21 pi. The only macroscopic finding was the enlarged spleen in both deceased and euthanized rats. Histopathological examination is in progress. Based on these results it seems that FTNF002-00 *Francisella tularensis* ssp. *holarctica* isolates are indeed more virulent than B.13 strains. We hypothesize that this difference in the virulence could be behind the different pathological picture observed in *Francisella tularensis* ssp. *holarctica* infected European brown hares at the western and central-eastern part of the continent.

S10-4

Analysis of *Francisella tularensis* strains isolated from humans and small rodents during year 2013 tularemia outbreak in Khanty-Mansiysk Sity, west Siberia

V. M. Pavlov[1], I. I. Kozlova[2], A. N. Mokrievich[1], O. D. Shutko[2], V. S. Timofeev[1], R. I. Mironova[1], T. S. Kuznetsova[2], N. M. Fayzullina[2], T. Y. Kudryavtseva[1], T. I. Kombarova[1], I. A. Dyatlov[1]

[1] State Research Center for Applied Microbiology and Biotechnology, [2] Center of Hygiene and Epidemiology in the KMAO-Yugra; Obolensk, Khanty –Mansiysk, Russia

In late summer 2013 in Khanty-Mansiysk and its suburban area there was a sharp aggravation of the epizootic situation on tularemia. In August-September 2013 fell ill with tularemia, according to official information, 1,005 people, including 156 children. Khanty-Mansi autonomous Area is located in the natural focus of tularemia floodplain swamp type. Vector mosquitoes and horseflies are that abundantly populate the area due to the peculiarities of its hydrography. Before this event, the last major outbreak in the region was recorded in 1983 - 1985's, when hundreds of people sick with tularemia. Almost all patients were congested with insect bite. Analysis of samples of biological material, selected from: a) pustules of District Hospital, Khanty-Mansiysk patients diagnosed with "tularemia"; b) small rodents caught in the territory of the district center and the surrounding area during the period from 08.23.2013 to 09.29.2013, was shown presence of F.tularensis tularemia. We analyzed 11 strains isolated from patients and from small rodents. Biological material was taken from seven patients. After spreading of pustul content on the FT-agar clones of tularemia microbe were detected after 4 days of culture growth. For bacteriological analysis were caught 24 rodents (15 red voles, shrews 7 and 2 gray house mouse). The causative agent of tularemia was detected in 4 of the 24 analyzed rodent spleens. All isolates from humans and rodents contain, according to PCR analysis, *F. tularensis* species-specific iglC gene, amplicon size distribution with Chi1f primer were identical to the spectrum characteristic for *F. tularensis* subsp. *holarctica* and isolates were resistant to erythromycin. Phylogenetic relationship between new strains, as well as strains isolated previously from tularemia foci of Siberia, the Urals and Kazahstana was determined by VNTR-analysis. The type of studied tularemia microbe is Holarctic subspecies. Strains were genetically identical in VNTR loci 24 and differed only in the hypervariable locus Ft-M3. 50% of analyzed strains contained 15 repeats at locus Ft-M3.This genotype was found in strains isolated from humans, and from at shrews. In patients with tularemia were found genotypes also with 9 and 10 repeats. Three strains isolated from rodents had 17 repetitions in this locus. All strains are located in one cluster, common to strains previously identified in the Southern Urals and the Republic of Kazakhstan. Detection of *F. tularensis* strains from men and shrews with the same number of repetitions in Ft-M3 locus, may indicate a common source of infection in rodents and human.

S10-5

Gallium potentiates the antibacterial effect of gentamicin against *Francisella tularensis*

Helena Lindgren and Anders Sjöstedt

Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS); Umeå University, SE-90185 Umeå, Sweden

Gentamicin is among the recommended therapies against various facultative intracellular organisms, *e.g.*, *F. tularensis*. Studies have demonstrated synergistic effects between aminoglycosides and iron chelation against multiple Gram-negative and Gram-positive bacteria. Here, we show results, which supports that a continuous uptake of iron is a means of SCHU S4 to minimize the effects of gentamicin. Specifically, the extracellular iron concentrations were directly correlated to the susceptibility to gentamicin. Further proof of the intimate link between iron availability and antibiotic susceptibility was the finding that deletion mutants of SCHU S4, which were defective for iron uptake, showed enhanced gentamicin susceptibility. In view of this iron dependency of SCHU S4 to resist gentamicin toxicity, it was hypothesized that gallium could potentiate the effect of this antibioticum, since gallium is sequestered by iron uptake systems. The Ferrozine assay demonstrated that the presence of gallium inhibited > 70% of the iron uptake by SCHU S4. The possibility of a combined effect of gentamicin and gallium for growth inhibition of SCHU S4 was assessed in CDM, bone marrow derived macrophages and in mice. Collectively, the data demonstrates that SCHU S4 depended on iron to minimize the effects of gentamicin and that gallium, which inhibited the iron uptake, potentiated the bactericidal effect of gentamicin on SCHU S4 *in vitro* and *in vivo*.

S10-6

Qualification of cynomolgus macaque model of pulmonary tularemia

Lynda Lanning[1], Patrick Sanz[1], Larry Wolfraim[1], Christopher Houchens[2], Kristian Omland[3], Steven Heyse[1], Robert Johnson[1], Ed Nuzum[1], Judith Hewitt[1], Tina Guina[1]

[1] Division of Microbiology and Infectious Diseases (DMID), The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), USA; [2] Division of CBRN Countermeasures, Biomedical Advanced Research and Development Authority (BARDA), USA; [3] Stantec, South Burlington, VT, USA

The National Institute of Allergy and Infectious Diseases (NIAID) and the Biomedical Advanced Research and Development Authority (BARDA) formed a Working Group for qualification of the cynomolgus macaque (*Macaca fascicularis*) model of pneumonic tularemia as a Drug Development Tool with Food and Drug Administration (FDA) (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284078.htm). The Working Group intent is to qualify the natural history model of pneumonic tularemia in cynomolgus macaque based on the results of the LD_{50} and natural history studies that were completed at Battelle Biomedical Research Center (BBRC), Lovelace Respiratory Research Institute (LRRI) and The United States Army Medical Research Institute for Infectious Diseases (USAMRIID) in 2007-2015. The qualified model will provide basis for the treatment model for use in evaluating the effectiveness of antimicrobial drugs and therapeutics for the treatment and/or post-exposure prophylaxis of pneumonic tularemia. This presentation will include examples of data from LD_{50} and natural history studies and proposed cynomolgus macaque model parameters that are currently under discussion with the FDA including animal species, age and gender; challenge strain and cell banks characterization; challenge agent preparation and exposure methods; and primary and secondary endpoints.

Session 11:

Clinical Disease, Epidemiology, and Ecology of Tularemia

Session 11: Clinical Disease, Epidemiology, and Ecology of Tularemia

Chairs: Roland Grunow
Robert Koch Institute, Germany

Jeannine Petersen
CDC, USA

S11-1 Genomic mutations linked to virulence differences among *Francisella tularensis* A1 strains
Jeannine M. Petersen, Caroline Odling, Mats Forsman, Luke Kingry, Laurel Respicio-Kingry, Kiersten Kugeler, Claudia Molins, Dawn Birdsell, David M. Wagner, Paul Keim, Anders Johansson

S11-2 The unexpected is most fascinating – tularemia in a niche of Germany revealed a new subclade of clade B.12
Klaus Heuner & Christoph Schulze, Kerstin Myrtennäs, Edvin Karlsson, Knut Große, Peter Kutzer, Daniela Jacob, Mats Forsman and Roland Grunow

S11-3 Erythromycin resistance in *Francisella tularensis* is explained by a single point mutation that occurred only once during the evolution of the species
Edvin Karlsson, Adrian Lärkeryd, Igor Golovlev, Malin Granberg, Eva Larsson, Caroline Öhrman, Dawn Birdsell, Paul Keim, David M. Wagner, Marcin Niemcewicz, Mats Forsman, Anders Johansson

S11-4 Vector-borne epidemic (group disease) outbreaks of tularemia in Russian federation in XXI century
I.S.Meshcheryakova, T.N.Demidova, Kormilitsyna. T.V.Mikhailova

S11-5 A novel approach for modelling the geographical risk distribution of getting tularaemia and determining change over time
Xija Liu, Amelie Desvars, Anders Sjöstedt, Andres Johansson, Patrik Rydén

S11-1

Genomic mutations linked to virulence differences among *Francisella tularensis* A1 strains

Jeannine M. Petersen, Caroline Odling, Mats Forsman, Luke Kingry, Laurel Respicio-Kingry, Kiersten Kugeler, Claudia Molins, Dawn Birdsell, David M. Wagner, Paul Keim, Anders Johansson

Centers for Disease Control and Prevention, Fort Collins, Colorado, USA, Swedish Defence Research Agency (FOI), Umea, Sweden, University of Umea, Umea Sweden, Northern Arizona University, Flagstaff, Arizona, USA

Worldwide, two subspecies of *F. tularensis*, subsp. *tularensis* (type A) and subsp. *holarctica* (type B), cause human tularemia. Type A strains display a restricted distribution limited to North America, have higher virulence in animals, and cause more severe illness in humans as compared to type B strains. In the last decade, virulence differences have been discerned among type A subpopulations defined by *Pme*I PFGE. A1b strains are the most virulent, exhibiting the highest mortality in patients and shortest survival times in C57BL/6 mice. To decipher the genomic basis for virulence differences among A1 subpopulations, 106 *F. tularensis* A1 strains isolated from patients in the United States were assigned into one of the three subpopulations, A.I.3, A.I.8 or A.I.12, using canSNP Melt-MAMA PCR assays defined by whole genome phylogenies. Two strains from each of the three A.I subpopulations, A.I.3, A.I.8 and A.I.12, were genome sequenced using Illumina HiSeq 2000 paired-end and mate-pair libraries. Whole genomes were assembled and structural arrangement of draft closed genomes confirmed by PCR. Epidemiologic analysis of the 106 A.I strains in the context of the SNP typing data and clinical information revealed mortality for patients infected with A.I.3 and A.I.8 strains was significantly higher as compared to patients infected with A.I.12 strains, whereas mortality for patients infected with either A.I.3 or A.I.8 strains did not differ. Whole genome comparisons identified 38 SNPs, 1 deletion, 7 VNTRs and a 948 base pair inversion in the A.I.12 genomes as compared to the A.I.3 and A.I.8 genomes. In silico *Pme*I PFGE of the closed genomes established the 948 bp A.I.12 specific inversion results in *Pme*I restriction fragments with differing length, thereby elucidating the genomic basis for PFGE identification of type A1 subpopulations with differing clinical outcomes. Phylogenetic analyses of the A.I.3, A.I.8 and A.I.12 genomes, based on SNPs and structural arrangements, suggest virulence differences among A.I subpopulations arose as the result of a decrease in virulence along the A.I.12 branch.

S11-2

The unexpected is most fascinating – tularemia in a niche of Germany revealed a new subclade of clade B.12

Klaus Heuner[1] & Christoph Schulze[2], Kerstin Myrtennäs[3], Edvin Karlsson[3], Knut Große[4], Peter Kutzer[2], Daniela Jacob[1], Mats Forsman[3] and Roland Grunow[1]*

[1] Centre for Biological Threats and Special Pathogens (ZBS 2), Robert Koch Institute, Nordufer 20, 13353 Berlin, Germany; [2] Landeslabor Berlin-Brandenburg, Frankfurt (Oder), Germany; [3] CBRN Defence and Security, Swedish Defence Research Agency (FOI), Umea SE-90182, Sweden; [4] Gesundheits- und Veterinäramt Stadt Brandenburg, Klosterstraße 14, 14770 Brandenburg an der Havel, Germany

Tularemia in Germany is a re-emerging zoonotic disease and recently an unexpected high seroprevalence was detected by us and others in wild animals in the German federal State of Berlin/Brandenburg poorly investigated for tularemia before. For the first time, a beaver was found in Germany dead from tularemia. The results from histological, PCR-, and VNTR-analysis on the diseased beaver, as well as PCR-analysis on surrounding water indicate that the beaver had a systemic infection and contracted the disease in the nearby area where the tularemia bacteria persisted in the environment during the season. We hypothesized that signs of a recent introduction of, or an expansion of present *Francisella tularensis* strains, could be revealed by high-resolution genetic characterization of strains circulating in wild life. Therefore, we investigated diseased and hunted wild animals as well as water sites in the Berlin/Brandenburg region for the presence of *F. tularensis* and by full genome sequencing of the obtained *Francisella* isolates. Strains were further analyzed by VNTR typing, INDEL and canSNP analysis. The phylogenomic analysis of three recovered *F. tularensis* subsp. *holarctica* isolates identified them as members of the basal B.12 clade (Erythromycin resistant strains). The findings of a member of a new B.13 sister clade, B.76 (isolated from raccoon dog), the first German member of the B.39 subclade (isolated from red fox), and the first isolate from a beaver (subclade B.33) in Germany among the small set of investigated animals, indicate that the diversity in Germany is vastly underestimated and that tularemia is more frequent in wild life than so far detected. All together, the discovered diversity suggests a long presence of tularemia in East Germany allowing the development of a pool of different genomic variants of *F. tularenis*. In addition, we recently identified and described a first German *Francisella* isolate, not belonging to the species *F. tularensis*, indicating the presence of further unidentified *Francisella* species in Germany. The described findings indicate, that further investigations are necessary to receive a better knowledge about the occurrence and distribution of different *F. tularensis holarctica* isolates as well as about the presence of putative further *Francisella* species in Germany.

S11-3

Erythromycin resistance in *Francisella tularensis* is explained by a single point mutation that occurred only once during the evolution of the species

Edvin Karlsson[1], Adrian Lärkeryd[1], Igor Golovlev[2], Malin Granberg[1], Eva Larsson[1], Caroline Öhrman[1], Dawn Birdsell[3], Paul Keim[3], David M. Wagner[3], Marcin Niemcewicz[4], Mats Forsman[1], Anders Johansson[2]

[1] CBRN Defence and Security, Swedish Defence Research Agency, Umeå, Sweden; [2] Department of Clinical Microbiology, Clinical Bacteriology, and the Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Umeå, Sweden; [3] Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA; [4] Wojskowy Instytut Higieny i Epidemioligii, Biological Threat Identification and Countermeasure Center, Warsaw, Poland Address: CBRN Defence and Security, Swedish Defence Research Agency, Cementvägen 20, SE-90182, Umeå, Sweden; edvin.karlsson@foi.se

Francisella tularensis subsp. *holarctica* has been subdivided into biovar I (Ery^S) and biovar II (Ery^R) based on the ability of some strains to resist the macrolide antibiotic erythromycin. In other bacterial species the macrolide resistance phenotype may be caused by several distinct genetic changes occurring independently in different genetic lineages. It is therefore unclear how the biovar I and II division of *F. tularensis* relates to the current phylogenetic classification system based on clonal inheritance of canonical single nucleotide polymorphisms (canSNPs). Here, we investigate the molecular evolution of erythromycin resistance in *F. tularensis*. Three hundred fourteen *F. tularensis* subsp. *holarctica* strains were tested for susceptibility to erythromycin using disc diffusion and their genome sequences were analysed for mutations in three genes (*rrl*, *rplD*, and *rplV*) that were previously reported to cause resistance in other species. Using canSNPs retrieved from the genomes all strains were assigned to the current global phylogenetic classification system. There was a perfect correlation between biovar II and phylogenetic group B.12. Sequence analysis revealed that erythromycin resistant strains (n=195) but no other strains had a base substitution at position 2059 in the *rrl* gene encoding 23S rRNA – a mutation known to be involved in macrolide resistance in many other bacteria. The 2059 substitution was present in all three copies of *rrl* in every genome of a strain set representing genetic diversity within the B.12 group (n=25). We constructed a suicide vector containing the 2059 substitution, introduced the substitution in one of the three *rrl* copies in a *Francisella tularensis* subsp. *holarctica* strain susceptible to erythromycin, and confirmed that this mutation caused erythromycin resistance. In conclusion, a single nucleotide substitution in the rrl gene occurred once in an ancestor of the B.12 phylogenetic group of *F. tularensis* subspecies *holarctica* and has since been clonally inherited. The mutation causes the Ery^R phenotype of biovar II strains. All biovar II strains can be unequivocally assigned to the canSNP B.12 group in the existing phylogenetic classification framework. This is important as it allowed us to compare the geographical distribution of strains described in scientific publications wherein the two systems were used independently. This revealed that the B.12 group is the most common and geographically most widespread phylogenetic group in Eurasia.

S11-4

Vector-borne epidemic (group disease) outbreaks of tularemia in Russian federation in XXI century

I. S. Meshcheryakova, T. N. Demidova, Kormilitsyna. T. V. Mikhailova

N F.Gamaleya Federal Research Centre of Epidemiology and Microbiology, Moscow, 123098 Moscow, Gamaleya str.18, e-mail: meshis@mail.ru, Russia

Tularemia is a zoonosal infection with natural focality.Tularemia foci are widely distribution and occur epidemically over large parts of Russia. The most dangerous in epidemic sense are natural foci of tularemia of bottomland-swamp type. They are distinct in their stability defined by high ecological plasticity of the agent tularemia. It keeps its epizootic and epidemic potential through many years and decades. The disease incidences among people are provoked by epizootic outbreaks that appear in the populations of hypersensitive semiaquatic animals (mainly, the water vole). In recent years we could reveal some peculiarities of epidemiological manifestation of tularemia.Tularemia was not only as sporadic infection but as rather intensive epidemic outbreaks, over 75% of patients were engaged in urban areas. Mass disease incidents of tularemia in Russia have stopped after the immunization with live tularemia vaccine. However, epizootic outbreaks among wild animals continued. The analysis of the reasons of occurrence of vector-borne outbreaks of tularemia in the last decade (the Central Federal district, 2005; the Archangelsk region, 2010-2014; the town of Khanty-Mansiysk, 2013) was carried out. Sources of infection were small mammals and vector – bloodsucking insects such as horseflies, mosquitoes. Major epidemic outbreak of tularemia that involved 1005 people was registered in the town of Khanty-Mansiysk. The disease incidences among people are connected with their stay on the territory of the active natural focus of tularemia. The main clinical form was ulceroglandular, moderatry severe or mild course of the disease. Diagnosis was based on clinical and epidemiological data and confirmed by laboratory methods. Discussed data efficiency immuno-serological and molecular-genetic (PCR–RV) methods of laboratory diagnostics of tularemia. The research confirmed high epizootic activity of the natural focus: the cultures of the disease agent were isolated, antibodies were found in the blood of small mammals. The research showed that a lot of species of small mammals are involved in this process. The outbreak was stopped after vaccination and other measures. The vaccination was performed for 15 846 people during the outbreak. Recommendations for control over the epizootic and epidemic situation in the territory of the natural foci of tularemia to prevent epidemic outbreak (group disease) were given.

S11-5

A novel approach for modelling the geographical risk distribution of getting tularaemia and determining change over time

X. Liu[1,3], A. Desvars[1], A. Sjöstedt[1], A. Johansson[1], P. Rydén[2,3]

[1] Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, 901 85 Umeå, Sweden; [2] Department of Mathematics and Mathematical Statistics, Umeå University, 90187 Umeå, Sweden; [3] Computational Life Science Cluster (CLiC), Umeå University, 90187 Umeå, Sweden

Background: The aim was to develop statistical tools allowing us to construct high resolution disease maps, identify environmental factors associated with the risk of contracting tularemia, and study risk change over time. As a proof of concept three problems related to two endemic regions in Sweden were considered: to study if the amount of streaming water is associated with high risk, to identify rivers associated with high risk, and to compare the geographically expansion of tularemia over time (2000-2010) in the newly emerged endemic region Örebro and the since long established region Västerdalarna.
Methods: We consider a geographical defined endemic region for which we have disease case data (geo-coordinates for the place of contraction and the disease onset date) as well as population data. The region was divided into cells (l = 4.2 km) and for each cell the number of inhabitants and tularemia cases were estimated. Monte Carlo simulation was used to estimate the *exposure* (*i.e.* the number of people at risk of being infected). A smoothed risk map was estimated by using the cell data and the distribution of the *relative risk* was calculated by normalizing the smoothed risk.
Niche modeling was done by quantifying the ecological of interest and then testing if there was a significant correlation with the risk. The amount of streaming water was estimated by using the length of rivers crossing the cell. Furthermore, the risk in cells located nearby some specific rivers was compared to the risk in the rest of the region. A bootstrap test was developed to identify temporal differences in the distribution of the relative risk.
Results: Simulations showed that the suggested tests had reasonable sensitivity when the false positive rate was fixed at 5%. The sensitivity increased with the sample and effect size. For Västerdalarna the amount of streaming water was not associated with a high risk of contracting tularemia ($r=-0.02$, $p>0.05$), but within the region several rivers were spatially significantly associated with a high risk. The region Örebro had a significantly lower spatial expansion rate ($p<0.01$) than Västerdalarna and the expansion patterns differed between the regions.
Conclusion: We propose procedures to investigate the spatio-temporal distribution of tularemia cases within endemic regions. These procedures can be powerful tools for detecting niches and geographically defined areas associated with high risk. Furthermore, they allow us to study spatial risk patterns over time.

Meeting Attendees

Patricia Renesto
CNRS UMR5163
Institut Jean Roget
Domaine de la Merci
38042 Grenoble Cedex 9, France
+330476637484
patricia.renesto@ujf-grenoble.fr

Sherry Kurtz
U.S. Food and Drug Administration
10903 New Hampshire Avenue
20903 Silver Spring
2404029655
sherry.kurtz@fda.hhs.gov

Roberto De Pascalis
CBER, Food and Drug Administration
10903 New Hampshire Avenue
20903 Silver Spring
2404029465
roberto.depascalis@fda.hhs.gov

Thomas Inzana
VATech, VMCVM
Life Sciences 1, 970 Washington Street, SW
24061 Blacksburg, USA
5402315188
tinzana@vt.edu

Wayne Conlan
National Research Council Canada
100 Sussex Drive
1105 Ottawa,Canada
16139938829
wayne.conlan@nrc-cnrc.gc.ca

Karen Elkins
CBER / FDA
10903 New Hampshire Avenue
Silver Spring, MD, USA
2404029507
karen.elkins@fda.hhs.gov

Aynur Karadenizli
Kocaeli University Medical *Faculty*
AD Umuttepe
41380 Kocaeli, Turkey
+905302570820
aynuryk2010@yahoo.com

Joshua Casulli
University of Manchester
Core Technology Facility, Grafton Street,
Manchester, M13 9PT
Manchester, United Kingdom
07920264439
joshua.casulli@postgrad.manchester.ac.uk

Anders Johansson
Umeå University
90185 Umeå, Sweden
+46703590830
anders.f.johansson@umu.se

Chinmay Dwibedi
Umeå University
Historiegrand 4b
90734 Umeå, Sweden
+46707871511
chinmay.dwibedi@climi.umu.se

Lenka Plzakova
University of Defense, Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
973255201
lenka.plzakova@unob.cz

Yvan Caspar
CHU, LAPM CNRS/UJF
Campus Santé
Grenoble, France
33476765479
ycaspar@chu-grenoble.fr

Max Maurin
CHU, LAPM CNRS/UJF UMR 5163
Campus Santé
Grenoble, France
33476765479
mmaurin@chu-grenoble.f

Marina Djordjevic Spasic
Clinical center Nis
Bulevar Zorana Djindjica 48
18000 Nis, Serbia
+381 184232221
marina djordjevic@yahoo.com

Joel Bozue
USAMRIID
1425 Porter Street
21702Fort Detrick, USA
3016194934
jaboz@comcast.net

Tina Guina
The National Institute of Health (NIH)
5601 Fishers Lane, 8G34
20892 Bethesda, USA
2406273266
tina.guina@nih.gov

Camille Robert
CNRS Université deToulouse
205 Route de Narbonne
31077 Toulouse, France
+33561176562
camille.robert@ipbs.fr

Leidy Lagos
University of Oslo, School of Pharmacy
Solstad Terrasse 50
1350 Oslo Norway
004741079225
l.x.l.rojas@farmasi.uio.no

Massimo Fabbi
IZSLER
Via A. Bianchi 9
Brescia, Italy
00390382422006
massimo.fabbi@izsler.it

Nadia Vicari
IZSLER
Via A. Bianchi 9
Brescia, Italy
+390382422006
nadia.vicari@izsler.it

Miklos Gyuranecz
Institute for Veterinary Medical Research,
CAR-HAS
Hungaria krt. 21..
1143 Budapest, Hungary
+36302777305
m.gyuranecz@gmail.com

Pavel Rehulka
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420973255199
pavel.rehulka@unob.cz

Zsuzsa Kreizinger
Institute for Veterinary Medical Research,
Centre for Agricultural Research,
Hungarian Academy of Sciences
H-1143 Budapest, Hungária krt.21
Budapest, Hungary
+3614674060
zsuzsakreizinger@yahoo.com

Annika Posautz
Research Institute of Wildlife Ecology,
Savoyenstrasse 1,
1160 Vienna, Austria
Annika.Posautz@vetmeduni.ac.at

Mark Wolcott
USAMRIID
1425 Porter Street
Ft Detrick MD 21773, USA
3016194738
mark.wolcott.civ@mail.mil

Nigou Jerome
CNRS- Université de Toulouse
205 route de Narbonne
Toulouse, France
33561175554
jerome.nigou@ipbs.fr

Joann Borjesson
National Veterinary Institute (SVA)
75189 Uppsala
75189 Uppsala, Sweden
+4618674257
joann.borjesson@sva.se

Yannick Brenz
Bernard Nocht Institute for Tropical
Medicine
Bernard-Nocht -Strasse 74
Hamburg, Germany
00494042818473
brenz@bni-hamburg.de

Marcia L. Merryman
Battelle Memorial Institute
4298 Basswood Road, Freeland, USA
5309024201
mlmerryman@ucdavis.edu

Eram Javed
Umeå University
Umeå 90187, Sweden
+46727005058
eram.athar2013@gmail.com

Lena Lindgren
Umeå University
Umeå 90185, Sweden
+46907851152
lena.lindgren@umu.se

Magdalena Proksova
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420775227659
magdalena.proksova@unob.cz

Maria Wijkander
Public Health Agency of Sweden
Folkhälsomyndigheten, 171 82 Solna
Stockholm, Sweeden
0046102052441
maria.wijkander@folkhalsomyndigheten.se

Iris Klein
Robert Koch-Institut
Nordufer 20
13353 Berlin, Germany
0049 30 18754 2103
KleinI@rki.de

Anne Barduhn
Robert Koch-Institut
Nordufer 20
13353 Berlin, Germany
0049 30 18754 2103
barduhna@rki.de

Borys Stegniy
National Scientific Center “Institute of Experimental and Clinical Veterina”
83, Pusgkinskaya St.
Kharkov, Ukraine
+38 (050) 3515048
pozdnyakovana@state.gov

David Wagner
Northen Arizona University
Center for Microbial Genetics and Genomics
Flagstaff, USA
9285230686
Dave.Wagner@nau.edu

Anders Sjostedt
Clinical Bacteriology
Umeå University
Umeå 90185, Sweden
+46907851120
anders.sjostedt@.umu.se

Kjell Eneslatt
Clinical Bacteriology
Umeå University
Umeå 90185, Sweden
+46907854659
kjell.eneslatt@climi.umu.se

Kun Sun
Clinical Bacteriology
Umeå University
Umeå 90185, Sweden
+46 90 7851819
sun.kun@climi.umu.se

Igor Golovliov
Clinical Microbiology
Umeå University
Umeå SE-90185, Sweden
+46 907 851 819
igor.golovlev@climi.umu.se

Roland Grunow
Robert Koch-Institut, ZBS 2
Nordufer 20
Berlin 13353, Germany
+49 30 18754 2100
GrunowR@rki.de

Klaus Heuner
Robert Koch Institute
Nordufer 20
Berlin 13353, Germany
heunerk@rki.de

Bai-Yu Lee
UCLA
10833 Le Conte Ave
Los Angeles 90095, USA
+3108259324
balee@mednet.ucla.edu

Sherry Mou
USAMRIID
1425 Porter Street, Fort Detrick
Frederick, USA
+(301)6194935
sherrymmou@yahoo.com

Kerstin Myrtennäs
Swedish Defence Research Agency
Cementvägen 20
Umeå University
Umeå 90185, Sweden
+46 90 106844
kerstin.myrtennas@foi.se

David Thanassi
Stony Brook University
242 Center for Infectious Diseases
Stony Brook, NY 11794-5120, USA
6319324549
david.thanassi@stonybrook.edu

Daniel Lee Clemens
University of California, Los Angeles
Le Conte Avenue 10833
Los Angeles 90095, USA
+3107947153
dclemens@mednet.ucla.edu

Miriam Maas
National Institute for Public Health and the Environment (RIVM)
Antonie van Leeuwenhoeklaan 9
Bilthoven 3720 BA, The Netherlands
+31(0)30 274 4712
miriam.maas@rivm.nl

Daniel Alford Powell
University of Arizona
1656 East Mabel Avenue
85724 Tucson, USA
+5206266447
danielpowell@email.arizona.edu

Susan Brock
University of Kansas Medical Center
3901 Rainbow Blvd.
Kansas City, KS 66160, USA
8163510349
sbrock@live.com

Iwaniak Wojciech
National Veterinary Research Institute
Al. Partyzantow 57
Pulawy 24-100, Poland
+48 81 889 30 20
iwaniakw@piwet.pulawy.pl

Amy Elizabeth Rohlfing
Boston Children's Hospital
300 Longwood Ave Enders 770.7
Boston, MA 02115, USA
7087521375
amy.rohlfing@childrens.harvard.edu

Rachael Claire Wilkinson
University of Southampton
Souhampton SO17 1BJ, United Kingdom
07980197005
rw2v11@soton.ac.uk

Patrik Ryden
Umeå University
901 87 Umeå, Sweden
+46704823555
patrik.ryden@.umu.se

Aimee L. Cunningham
University of Maryland Baltimore
685 W. Baltimore St. Rm 480
21201 Baltimore, MD, United States
4107064418
acunningham2@medicine.umaryland.edu

Eileen M. Barry
University of Maryland Baltimore
685 W. Baltimore St. Rm 480
21201 Baltimore, MD, United States
(410) 7065328
ebarry@medicine.umaryland.edu

Xijia Liu
Umeå University
Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS)
Umeå Sweden
46907851120
xijia.liu@umu.se

Vitaly Mikhailovich Pavlov
SRCAMB
Obolensk 142253, Russia
+4967360063
vitpav@obolensk.org

Irina S. Mesheryakova
N.F.Gamaleya Research Centre of Epidemiology and Microbiology
Gamaleya str.18
Moscow 123098, Russia
+74991936365
meshis@mail.ru

Daniela Jacob
Robert Koch Institut,
ZBS2
Nordufer 2
Berlin 13353, Germany
+4930187542934
JacobD@rki.de

Natalia Vladimirovna Pavlovich
Rostov-on-Don Research Institute for Plague Control
st. M. Gorkogo 117
Rostov-on-Don 344002, Russia
+79282797868
info@tularemia.ru

Monique L. van Hoek
George Mason University
10910 University Blvd
Manassas 20110, USA
7039934273
mvanhoek@gmu.edu

Izet Sadiku
University Clinical Center
Clinical Center of Kosovo
Prishtine, Kosovo
+37744124029
izadoc@yahoo.com

Elena Kulikalova
Irkutsk Antiplague Research Institute of Rospotrebnadzor
Trilissera 78
Irkutsk 664047, Russia
+73952220140
e.kulikalova@yandex.ru

Mats Forsman
Swedish Defence Research Agency (FOI)
Cementvägen 20
Umeå 901 82, Sweden
+46706180026
mats.forsman@foi.se

Alain Charbit
INSERM
Inserm U11151-CNRS UMR 8253
Paris 75015, France
33 656890536
alain.charbit@inserm.fr

Karl Erik Klose
One UTSA Circle
San Antonio TX 78248, USA
2104586140
Karl.Klose@utsa.edu

Gvantsa Chanturia
NCDC
#9, Asatiani Street
Tbilisi, Georgia
00995322398946
stkhelia@ch2m.com

Irma Burjanadze
NCDC
#9, Asatiani Street
Tbilisi, Georgia
00995322398946
stkhelia@ch2m.com

Tsiuri Tushishvili
NCDC
#9, Asatiani Street
Tbilisi, Georgia
00995322398946
stkhelia@ch2m.com

Tamar Chikviladze
NCDC
#9, Asatiani Street
Tbilisi, Georgia
00995322398946
stkhelia@ch2m.com

Revaz Solomonia
Ilia State University
Kakutsa Cholokashvili Ave 3/5
Tbilisi, Georgia
00995322220009
stkhelia@ch2m.com

Maia Meparisvili
Ilia State University
Kakutsa Cholokashvili Ave 3/5
Tbilisi, Georgia
00995322220009
stkhelia@ch2m.com

Petra Oyston
DSTL Porton Down
Salisbury, Wiltshire, United Kingdom
+441980 613641
PCOYSTON@mail.dstl.gov.uk

Herbert Tomaso
Friedrich-Loeffler-Institut, Federal
Research Institute for Animal Health
Naumburgerstrasse 96a,
07743 Jena, Germany
+4936418042243
herbert.tomaso@fli.bund.de

Athar Alam
Umeå University
Department of Clinical Bacteriology
90187 Umeå, Sweden
+46767106466
athar.alam@climi.umu.se

Jiri Stulik
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420973253220
jiri.stulik@unob.cz

Thomas Henry
Inserm Lyon university
Centre International de Recherche en
Infectiologie
CIRI, Inserm U1111, 21 avenue Tony
Garnier, France
+33 (0)699176434
thomas.henry@inserm.fr

Ivona Pávková
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420 973 255 201
ivona.pavkova@unob.cz

Mateja Ozanic
Faculty of Medicine, University of Rijeka
Brace Branchetta 20
Rijeka 51000, Croatia
+38551651144
matejaozanic@gmail.com

Valentina Marecic
Faculty of Medicine, University of Rijeka
Brace Branchetta 20
Rijeka 51000, Croatia
+38551651144
valentina.marecic@medri.uniri.hr

Marina Santic
Faculty of Medicine, University of Rijeka
Brace Branchetta 20
Rijeka 51000, Croatia
+38551651267
marina.santic@medri.uniri.hr

Helena Lindgren
Clinical Bacteriology
Umeå University
Umeå 90185, Sweden
+46702294582
helena.e.lindgren@.umu.se

Simon Dove
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02115, USA
6179192888
simon.dove@childrens.harvard.edu

Ivo Fabrik
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420 973 255 199
ivo.fabrik@unob.cz

Lucie Balonova
Faculty of Military Health Sciences,
University of Defence
Trebesska 1575, Hradec Kralove 500 01,
Czech Republik
+420 973253223
lucie.balonova@unob.cz

Link Marek
Faculty of Military Health Sciences,
University of Defence
Trebesska 1575, Hradec Kralove 500 01,
Czech Republik
+420 973255199
link.marek@unob.cz

Monique Barel
INSERM U1002
156 rue de Vaugirard
Paris 75993, France
+33172606515
monique.barel@inserm.fr

Jana Klimentova
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420 973 255 201
jana.klimentova@unob.cz

Jason Huntley
University of Toledo College of Medicine
3000 Arlington Avenue, HEB259A
Toledo, OH 43614, USA
4193835456
jason.huntley@utoledo.edu

Bernard Arulanandam
University of Texas at San Antonio
One UTSA Circle
San Antonio, TX 78249, USA
2104586569
bernard.arulanandam@utsa.edu

Ales Macela
University of Defence
Trebesska 1575
Hradec Kralove 500 01, Czech Republic
+420 973 253 094
amacela@seznam.cz

Isabel Lopes de Carvalho
National Institite of Health
Av. Padre Cruz
Lisboa, Portugal
+351919042474
Isabe.lcarvalho@insa.min-saude.pt

Zuzana Krocova
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420 603905116
zuzana.krocova@unob.cz

Valeria Sheshko
FMHS, UoD
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420973255203
sheshko@pmfhk.cz

Marcus Aaron Horwitz
UCLA
Dept. of Medicine, 37-121
3102060074
mhorwitz@mednet.ucla.edu

Qingmei Jia
UCLA School of Medicine
37-121 Center for the Health Sciences,
10833 Le Conte Avenue
90095 Los Angeles, USA
qjia@mednet.ucla.edu

Catharine Bosio
RML/NIAID/NIH
903 South 4th street
Hamilton, USA
4063639425
bosioc@niaid.nih.gov

Klara Kubelkova
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420973255193
klara.kubelkova@unob.cz

Marie Elisabeth Honn
Umeå University
SE 91832 Savar, Sweden
0046 90 785 8787
marie.honn@umu.se

Daniela Putzova
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
00420973253269
daniela.putzova@unob.cz

Kerstin Rydzewski
Robert Koch-Institut
Nordufer 20
Berlin, Gremany
004930187542454
rydzewskik@rki.de

Hanne C. Winther-Larsen
University of Oslo
PO Box 1068 Blindern
0316 Oslo, Norway
+4722857011
hannewi@farmasi.uio.no

Caroline Öhrman
Swedish Defence Research Agency
Cementvagen 20
Umeå, Sweden
+46725709230
caroline.ohrman@foi.se

Kathryn M. Ramsey
Boston Childrens Hospital
300 Longwood Ave Enders 750.7
02115 Boston USA
6179192885
Kathryn.Ramsey@childrens.harvard.edu

Erik Kooping
Stony Brook University
Center for Infectious Disease
Stony Brook, USA
16316324231
erik.kopping@stonybrook.edi

Jeannine Petersen
US Centers for Disease Control
3156 Rampart Road
Fort Collins, USA
19702663524
jpetersen@cdc.gov

Laila Noppa
FOI, Swedish Defence Research Agency
Stenhagsvagen 5
Tavelsjo, Sverige
+46725709295
laila.noppa@foi.se

Javed Muhammad
University of Veterinary an Animal
Sciences
Outfall road
Lahore, Pakistan
0923219898223
javedM81@hotmail.com

Nadia Schuerch
Spiez Laboratory
Austrasse 3700
Spiez, Switzerland
+41584681401
nadia.schuersch@babs.admin.ch

Juraj Lenco
University of Defense
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove 50001, Czech Republic
+420973253223
lenco@pmfhk.cz

Charlotte Hall
DSTL
Dstl Port Down
Salisburry, UK
01980613993
CHALL@dstl.gov.uk

Vienna Brown
Colorado State University
Colorado State University-163 Campus Delivery
Fort Colins, CO
United States
9704917349
Vienna. Brown@colostate.edu

Laura Marshall
DSTL
Dstl Port Down
Salisburry, UK
01980614604
LEMARSHALL@dstl.gov.uk

Ruben Danielyan
NCDCP, MOH
TBD
Yerevan, Armenia
2813358000
hgarett@tti-corp.com

Ara Poghosyan
SFSS, MOA
TBD
Yerevan, Armenia
2813358000
hgarett@tti-corp.com

Arman Badalyan
CARD
TBD
Yerevan, Armenia
2813358000
hgarett@tti-corp.com

Armen Vardanyan
SFSS, MOA
TBD
Yerevan, Armenia
2813358000
hgarett@tti-corp.com

Paola Pilo
Institute of Veterinary Bacteriology
Laenggassstrasse 122
Bern, Switzerland
+41316312369
paola.pilo@vetsuisse.unibe.ch

Carl Gelhaus
MRIGlobal
425 Volker Blvd
Kansas City, USA
18163265068
cgelhaus@mriglobal.org

Girish Kirimanjeswara
Penn State University
W205 Millennium Science Complex
University Park, USA
18148635250
gsk125@psu.edu

Todd Kijek
USAMRIID
1425 Porter Street
Ft Detrick, USA
3016198256
todd.m.kijek.mil@mail.mil

Tom Kawula
University of North Carolina
Microbiology and Immunology
Chapel Hill, NC 27599, USA
0019199669699
kawula@med.unc.edu

Dag Mohlkert
Stickholm South Hospital, Department of radiology
Sjukhusbacken 10,
11883 Stockholm, Sweden
4686163359
dag.mohlkert@sodersjukhuset.se

Lauren Radinski
UNC-Chapel Hill
125 Mason Farm Road
Chapel Hill, USA
6303387513
lrad@email.unc.edu

Suman Mukhopadhyay
NIH/NIAD
5601 Fishers Lane
Bethesda, USA
2404232666
sm226a@nih.gov

Sharon Taft-Benz
University of North Carolina
125 Manson Farm Road
Chapel Hill, NC, USA
9199662637
sharon_taft-benz@med.unc.edu

Lyudmila Niazyan
CH2M Armenia office
10, Vazgen Sargsyan str.
Yerevan, Armenia
37441004091
Lyudmila.Niazyan@ch2m.com

Siobhan Cowley
FDA
10903 New Hampshire Ave
Silver Spring, USA
2404027153
siobhan_cowley@yahoo.com

Maria Alexandrovna Sukhova
State Research Center of Applied Microbiology and Biotechnology
Obolensk 142279
Moscow Region
Russian Federation
+79055207952
marik_suharikelf@mail.ru

Bradley Jones
University of Iowa
51 Newton Road, 3-330 Bowen Science Bldg.
Iowa City, USA
3193535457
bradley-jones@uiowa.edu

Azad Jafarov
Khacham, Anti Plague Division
Khachmaz, Azerbaijan
994502457424
zismayilov@tti-corp.com

Bakhtiyar Aydinov
Khachmaz Anti Plague Division
Khachmaz, Azerbaijan
994502457424
zismaylov@tti-corp.com

Kitty Chase
USAMRIID
1425 Porter St
Frederick MD, USA
3016190913
catherine.j.chase.ctr@mail.mil

Fatmire Kollçaku
National Institute of Public Health of Kosova
30000 Peja, Republic of Kosova
+37744142744
fatmiremk@gmail.com

Vanina Gafuri
National Institute of Public Health,branch Peja
30000 Peja, Republic of Kosova
+37744328470
vaninagafuri@hotmail.com

Shqipe Krasniqi
National Institute of Public Health,branch Peja
30000 Peja, Republic of Kosova
+37744364370
shqipe_krasniqi123@hotmail.com

Gavin Braunstein
DTRA
8725 John J Kingman Rd, MSC 6201
Fort Belvoir, USA
1-703-767-7788
gavin.m.braunstein.civ@mail.mil

Dawn Birdsell
Northern Arizona University
1395 S. Knoles, Bldg 56, 3rd Flr
Flagstaff, USA
9285230552
dawn.birdsell@nau.edu

Ofer Cohen
IIBR
Rishon-LeZion, ISRAEL
972-50-6234254
ofercohen777@gmail.com

Erez Bar-Haim
IIBR
POB 19, Ness Ziona, Israel
+972544715351
bh.erez@gmail.com

Liat Bar-On
Reuven-Lerer 24 Ness-Ziona, Ness ziona
Israel
liatgree@yahoo.com

Ehsan Mostafavi
Department of Epidemiology
Pasteur Institute of Iran
Tehran, Iran
00982166954320
mostafaviehsan@gmail.com

Yousef Abu Kwaik
Department of Microbiology and
Immunology &
Center for Predictive Medicine
Uinversity of Louisville Health Sciences
Center
Clinical and Translational Research Bldg.
Room 608
501 S. Hancock street
Louisville, KY 40202
Phone # (502) 852-4117 (office), (502)
852-4118 (lab)
abukwaik@louisville.edu

Abdolrazagh Hashemi Shahraki
Department of Epidemiology
Pasteur Institute of Iran
Tehran, Iran
0098-2166496448
abdolrazaghh@gmail.com

Abstracts Indexed by Author

Notes:

Notes:

Notes:

Notes:

Edition:

Book of Abstracts of 8th Internatonal Conference of Tularemia
organized by
Tularemia International Society

Editor:

Marina Santic

Publisher:

Faculty of Medicine, University of Rijeka

Printed by:

Recedo d.o.o., Zagreb

A CIP catalogue record for this book is available from the
University Library in Rijeka under 130722025

ISBN 978-953-7957-36-0