9th International Conference on Tularemia

16-19 OCTOBER, 2018

Centre Mont Royal, Montreal, Canada

TULAREMIA INTERNATIONAL SOCIETY

Grants & Sponsors

U.S. Food & Drug Administration

Funding for this conference was made possible in part, by the Food and Drug Administration through grant 1 R13 FD006469-01. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

PAUL G. ALLEN SCHOOL FOR GLOBAL ANIMAL HEALTH
About the Allen School

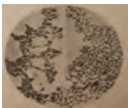
Paul G. Allen School at Washington State University graciously sponsored the opening session and reception

National Research Council Canada
Conseil national de recherches Canada

National Research Council Canada graciously sponsored the Final Program/Abstract books.
www.nrc-cnrc.gc.ca

The University of Texas at San Antonio graciously sponsored the Conference Bags.

MRIGlobal graciously sponsored Student Travel Awards.



TULAREMIA INTERNATIONAL SOCIETY

TULISOC - Tularemia International Society
www.tularemia-network.com

Dear Colleagues,

The organising committee welcomes you to the 9th International Conference on Tularemia in Montreal in La Belle Province of Quebec. The official motto of the province is “Je me souviens,” literally translated “I remember,” and we hope that you too will remember this conference long after its closing. Although Canada may not be an obvious hot spot for tularemia, interestingly, several Cree nations in northern Quebec show very high seroconversion rates, presumably because of their hunter-gatherer lifestyle. This is supported by serological surveys of the surrounding wildlife which also show serological evidence of exposure to *Francisella tularensis*. Indeed, according to these and other studies, *F. tularensis* appears to be the most predominant zoonotic bacterium in the wild in Canada. These are important reminders that in many parts of the northern hemisphere, the risk of tularemia remains a reality. The observations in people from northern Quebec remind us too that much remains unknown regarding the bacterium’s ecology, epidemiology, diagnosis, modes of transmission, and host-pathogen interactions. Moreover, countermeasures are still needed, especially in parts of the world where clinical capabilities are far from optimal. Thus, the scientific tularemia community is a relevant as ever, and at the 9th International Conference, we look forward to hearing from you about your latest findings. We sincerely hope that you have a productive meeting and have time to savour the delights of the host city renowned for its European-style buildings, great food, festivals, and museums.

On behalf of the Scientific Committee, TULISOC and Co-Chairs,

Wayne Conlan
President, TULISOC
National Research Council Canada, Ottawa, Canada

Karen Elkins
Center for Biologics Evaluation and Research, U.S. FDA, Silver Spring, MD, USA

Katy Bosio
NIAID, Rocky Mountain Labs, Hamilton, MT, USA

// Acknowledgements

International Organizing Committee:

Karen Elkins, CBER/FDA, USA
Catharine (Katy) Bosio, NIAID/NIH, USA
Lee-Ann Allen, University of Iowa, USA
Wayne Conlan, National Research Council Canada (*ex officio* as TULISOC President)
Roberto De Pascalis, CBER/FDA, USA
Jason Huntley, University of Toledo, USA
Tom Kawula, Washington State University, USA
Karl Klose, University of Texas-San Antonio, USA
Max Maurin, Université Grenoble Alpes, France
Petra Oyston, Defence Science and Technology Laboratory, United Kingdom
Jeannine Petersen, Centers for Disease Control, USA
Marina Šantić, University of Rijeka, Croatia
Jiri Stulik, University of Defence, Czech Republic

Session Chairs:

Lee-Ann Allen, University of Iowa, USA
Catharine Bosio, NIAID/NIH, Rocky Mountain Laboratories, USA
Simon Dove, Boston Children's Hospital, Harvard Medical School, USA
Raquel Escudero, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Spain
Thomas Henry, CIRI, International Center for Infectiology Research, France
Selçuk Kiliç, Public Health Institution of Turkey, Turkey
Karl Klose, University of Texas-San Antonio, USA
Kiersten Kugeler, Centers for Disease Control and Prevention, Fort Collins, USA
Helena Lindgren, Umeå University, Sweden
Max Maurin, CHU Grenoble Alpes, Université Grenoble Alpes, France
Petra Oyston, DSTL, United Kingdom
Marina Šantić, University of Rijeka, Croatia
Anders Sjöstedt, Umeå University, Sweden
Terry Wu, University of New Mexico Health Sciences Center, USA

Tularemia International Society Executive Council

President
Wayne Conlan, National Research Council Canada, Canada
Secretary
Karen Elkins, CBER/FDA, USA
Treasurer
Karl Klose, University of Texas-San Antonio, USA

Past President
Siobhán Cowley, CBER/FDA, USA

Congress Website: www.2018.tularemia.org

TULISOC Membership:

Any professional who is involved in research, teaching and/or public service and whose activities are related to the study of tularemia is eligible for membership provided that he/she supports the goals and objectives of the Society as set forth in the Terms of Reference and By-Laws. In addition, any graduate or undergraduate student who is involved in advanced training in any area related to the study of tularemia is eligible for student membership.

The Society shall consist of four types of members: regular, student, special, and emeritus.

Regular Member: $40 per year / $100 3 years
Any professional who is involved in research, teaching and/or public service and whose activities are related to the study of tularaemia is eligible for membership provided that he/she supports the goals and objectives of the Society as set forth in the Terms of Reference and By-Laws. Regular members may vote and hold office in the Society.

Student Member: $20 per year
Any graduate or undergraduate student who is involved in advanced training in any area related to the study of tularemia is eligible for student membership. The application form and each annual dues form must be signed by the student's advisor or department head. Student membership requires that mentors or sponsors are active members of TULISOC. Student membership shall terminate at the end of the calendar year in which student status terminates, at which time such individuals become eligible for regular membership according to the same criteria and procedures that apply to other scientists. Student membership dues will be at a reduced rate to be determined by the Executive Committee. Student members may not vote or hold office.

Emeritus Member
Regular and Special members who are retired from their primary positions may apply for Emeritus membership. Emeritus members enjoy the same privileges accorded to regular members but are not required to pay dues. Please contact the TULISOC to request Emeritus Membership.

Special Member
Scientists qualified for regular membership but unable, owing to economic or political conditions in their countries of residence, to pay regular dues. Applications for this category of membership shall be judged by the Executive Committee on a case-by-case basis, with respect to published lists of developing and economically disadvantaged nations. Dues of Special Members may be paid on a voluntary basis by other members of TULISOC, or may be waived by the Executive Committee. Special members may vote and hold office in the Society. Please contact the TULISOC to request Special Membership.

Members of TULISOC will benefit from a discount on registration fees for Tularemia conferences organised by the Society.

9th International Conference on Tularemia Program

Montreal, Canada
October 16 – 19, 2018

Breakfast, for those staying at the Best Western Ville-Marie, daily from ~ 6:30 – 8:00 AM

All meeting sessions will be at the Centre Mont-Royal
Lunch will be provided for attendees at the Centre Mont-Royal
Late afternoon poster sessions will include a cash bar

TUESDAY, OCTOBER 16	
17:00 – 19:30	**Welcome Reception** at McGill Faculty Club, *sponsored by: Paul G. Allen School for Global Animal Health at Washington State University*
18:00	**Keynote address** Wayne Conlan, NRC–Canada President, TULISOC (K-1) Biothreat Agents: Weapons of Mass Distraction?

WEDNESDAY, OCTOBER 17 All meeting sessions will be in the Centre Mont-Royal - Salon Cartier (I & II)	
8:30 – 11:50	**Session 1: Host Response and Immunity** **Co-chairs:** **Catharine Bosio**, RML, NIAID/NIH, USA **Thomas Henry**, CIRI, INSERM, France
8:30 – 8:40	**Katy Bosio**, Rocky Mountain Laboratories, NIAID/NIH, USA Session and Topic Overview
8:40 – 9:00	**Klara Kubelkova**, University of Defence, Faculty of Military Health Sciences *Francisella* inside the cell: implications for innate immune recognition of intracellular bacteria (S1-1)
9:00 – 9:20	**Joshua Casulli**, University of Manchester, United Kingdom CD200R limits the neutrophil niche for *Francisella tularensis* infection (S1-2)
9:20 – 9:40	**Forrest Jessop**, Rocky Mountain Laboratories, NIAID/NIH, USA Virulent but not attenuated *Francisella tularensis* resists IFN-g dependent antimicrobial responses through manipulation of host macrophage mitochondrial function (S1-3)

9:40 – 10:00	**Thomas Henry**, CIRI, INSERM, France Novel insights into inflammasomes responses to *Francisella* (S1-4)
10:00 – 10:30	Coffee Break
10:30 – 10:50	**Karen Elkins**, CBER/FDA *Francisella*-immune T lymphocytes do not alter intramacrophage bacterial trafficking but limit replication and cell-to-cell spread of *F. tularensis* LVS (S1-5)
10:50 – 11:10	**Lydia Roberts**, Rocky Mountain Laboratories, NIAID/NIH, USA (S1-6) Temporal and functionally distinct roles for resident and circulating T cells during vaccine-mediated protection against tularemia
11:10 – 11:30	**Girish Kirimanjeswara**, Penn State University Macrophage selenoproteins restrict intracellular replication of *Francisella tularensis* (S1-7)
11:30 – 11:50	**Jérôme Nigou**, Université de Toulouse, France Evaluation of the therapeutic potential of an anti-inflammatory mannodendrimer in a mouse model of *Francisella tularensis* infection (S1-8)
11:50 – 12:30	**Poster pitches for Poster Session #1** **Chair, Petra Oyston**, DSTL, United Kingdom
12:30 – 13:15 13:15 – 14:00	Lunch at the Centre Mont-Royal - Salon International (I & II) TULISOC Meeting **Chair, Wayne Conlan**, TULISOC President
14:00 – 16:30	**Session 2: Vaccines** **Co-chairs:** **Karl Klose,** Univ. Texas-San Antonio, USA **Terry Wu**, Univ. of New Mexico, USA Helena Lindgren, University of Umea, Sweden s
14:00 – 14:10	**Terry Wu**, University of New Mexico, USA Session and Topic Overview
14:10 – 14:30	**Doug Reed**, University of Pittsburgh, USA Small particle aerosols of *Francisella tularensis*; characterization and optimization to support pivotal animal efficacy studies (S2-1)
14:30 – 14:50	**Roberto De Pascalis**, CBER/FDA, USA Evaluation of the Ft-ΔClpB vaccine using working immune correlates predicts protection against *Francisella* challenge (S2-2)
14:50 – 15:10	**Petra C.F. Oyston**, DSTL, United Kingdom A protective subunit vaccine against tularaemia: delivery of antigens by glucan particles induces protection in rats (S2-3)

15:10 – 15:30	**Chandra Shekhar Bakshi**, New York Medical College, USA Evaluation of a plug and play approach for the development of multi-antigen subunit vaccines (S2-4)
15:30 – 15:50	**Xhavit Zogaj**, The University of Texas at San Antonio, USA Engineering OAg^{FTT} expression in *F. novicida* (S2-5)
15:50 – 16:10	**Sherry Kurtz**, CBER/FDA, Silver Spring, USA Options in the United States for licensing new biological products for rare infectious diseases (S2-6)
16:10 – 16:30	**Robert House**, Ology Bioservices, Frederick, USA Making the Cut: Considerations for Moving Candidates from Discovery to Advanced Development (S2-7)
16:30 – 18:30	**Poster Session #1**, with refreshments P1-01 – P1-42
THURSDAY, OCTOBER 18	
8:30 – 12:00	**Session 3: Pathogenesis and Cell Biology** **Co-chairs:** **Lee-Ann Allen**, University of Iowa, USA **Marina Šantić**, University of Rijeka, Croatia
8:30 – 8:40	**Marina Šantić**, University of Rijeka, Croatia Session and Topic Overview
8:40 – 9:05	**Lee-Ann Allen**, University of Iowa, USA F. tularensis-infected neutrophils are Trojan Horses for macrophage infection (S3-1)
9:05 – 9:30	**Briana Zellner,** University of Toledo, USA A *Francisella tularensis* LD-carboxypeptidase is required for virulence (S3-2)
9:30 – 9:55	**Jason Huntley,** University of Toledo, USA The *Francisella* tularensis γ-glutamyl cyclotransferase is required for virulence (S3-3)
9:55 – 10:25	Coffee Break
10:25 – 10:50	**Jiri Stulik**, Faculty of Military Health Sciences, Czech Republic Inactivation of *Francisella tularensis* gene encoding putative flippase has a pleiotropic effect upon production of various glycoconjugates: evidence for PilA protein modification by O-antigen (S3-4)

10:50 – 11:15	**Hannah Ledvina**, University of Washington School of Medicine, USA A phosphatidylinositol 3-kinase effector alters phagosomal trafficking to promote intracellular growth of *Francisella* (S3-5)
11:15 – 11:40	**Peter Benzige**r, Stony Brook University, USA Identifying virulence factors secreted by *Francisella tularensis* during infection (S3-6)
11:40 – 12:05	**Joseph Horzempa**, West Liberty University, USA Hidden in plain erythrocyte (S3-7)
12:05 – 12:45	**Poster pitches for Poster Session #2** **Chair, Jeannine Petersen**, CDC, USA
12:45 – 13:45	Lunch at the Centre Mont-Royal - Salon International (I & II)
13:45 – 16:00	**Session 4: Bacteriology and Gene Regulation** Co-chairs: **Petra Oyston**, DSTL, United Kingdom **Simon Dove,** Harvard University, USA
13:45 – 13:55	**Simon Dove**, Harvard University, USA Session and Topic Overview
13:55 – 14:20	**Maj Brodmann**, University of Basel, Switzerland The role of the dynamic Type VI Secretion System in *Francisella* virulence (S4-1)
14:20 – 14:45	**Tyler Goralski**, Pennsylvania State University, USA Plan B: Identifying a backup ribosome rescue system in *Francisella tularensis* (S4-2)
14:45 – 15:10	**David Thanassi**, Stony Brook University, USA Metabolic control of outer membrane vesicle and tube formation by *Francisella novicida* (S4-3)
15:10 – 15:35	**Patricia Renesto**, CHU Grenoble Alpes, Université Grenoble Alpes, France Structural and functional analysis of a putative lysine decarboxylase found in *Francisella* spp. (S4-4)
15:35 – 16:00	**Meenakshi Malik**, Albany College of Pharmacy and Health Sciences Understanding the mechanisms of oxidative stress responses and virulence of *Francisella tularensis* LVS (S4-5)
16:00 – 18:00	**Poster Session #2**, with refreshments P2-01 – P2-40

FRIDAY, OCTOBER 19	
8:30 – 12:00	**Session 5: Human Infection and Treatment** **Co-chairs:** **Max Maurin**, Univ. Grenoble-Alpes, France **Anders Sjöstedt**, Umeå University, Sweden
8:30 – 8:40	**Max Maurin**, CHU Grenoble Alpes, Université Grenoble Alpes, France Session and Topic Overview
8:40 – 9:05	**Roland Grunow,** Robert Koch Institute, Germany An uncommon outbreak of tularemia after a wine grape harvest in fall 2016, Germany, and molecular identification of the outbreak 'strain' as well as of the source of infection (S5-1)
9:05 – 9:30	**Christina Nelson**, Centers for Disease Control and Prevention, USA Transmission of *Francisella tularensis* by solid organ transplantation (S5-2)
9:30 – 9:55	**Yvan Caspar,** CHU Grenoble Alpes, Université Grenoble Alpes, France Tularemia in France: a ten-year overview and unusual clinical aspects (S5-3)
9:55 – 10:25	Coffee break
10:25 – 10:50	**Claire Siebert,** CHU Grenoble Alpes, Université Grenoble Alpes, France Mutations targeting the FupA/B lipoprotein of *F. tularensis* LVS exposed to ciprofloxacin open a new way for fluoroquinolone resistance linked to OMV secretion and biofilm formation (S5-4)
10:50 – 11:15	**Monique van Hoek**, George Mason University, USA Antimicrobial peptides against *Francisella tularensis* (S5-5)
11:15 – 11:40	**Deanna Schmitt,** West Liberty University, USA Identification of potential targets of resazomycins, a novel family of antibiotics against *Francisella tularensis* (S5-6)
11:40 – 12:00	**Elizabeth Dietrich**, Centers for Disease Control and Prevention Complete genome sequences and comparative analysis of the novel pathogen *Francisella opportunistica* (S5-7)
12:00 – 13:00	Lunch at the Centre Mont-Royal - Salon International (I & II)

13:00 – 16:00	**Session 6: Epidemiology and Ecology** **Chair:** **Kiersten Kugeler**, Centers for Disease Control and Prevention, USA **Raquel Escudero**, Instituto de Salud Carlos III, Spain
13:00 – 13:20	**Mats Forsman**, University of Umea, Sweden Session and Topic Overview
13:20 – 13:40	**Luke Kingry**, Centers for Disease Control and Prevention, USA Whole genome sequencing of *F. tularensis* from cultivable and non-cultivable sources to identify the origin of human cases of tularemia (S6-1)
13:40 – 14:00	**Maëllys Kevin**, Université Paris-Est/ANSES, France Whole-genome sequencing and molecular approaches for epidemiological surveillance and tracking of tularemia in France (S6-2)
14:00 – 14:20	**Isabel Lopes de Carvalho**, National Institute of Health, Portugal Genome-scale comparison of *Francisella tularensis* strains isolated in an endemic region of Spain (S6-3)
14:20 – 14:50	Coffee Break
14:50 – 15:10	**David Sundell**, Swedish Defence Research Agency, FOI, Sweden Complete *Francisella genomes* generated by nanopore sequencing directly from human and animal clinical specimens (S6-4)
15:10 – 15:30	**David Wagner**, Northern Arizona University, USA Type A.II strains of *Francisella tularensis* are quite capable of causing human disease and may be dispersed by wind and persist in the environmental in a quiescent state (S6-5)
15:30 – 15:50	**Natalie Kwit**, Vermont Department of Health, USA Northern trajectory of human tularemia — United States, 1965–2014 (S6-6)
18:30 – 21:30	**Tularemia 2018 Banquet** Awards, Future Meeting Plans, and Closing Program Vieux Port Steakhouse, Old Montreal

Meetings in CMR Salon Cartier (I & II) – Lunches in CMR Salon International (I & II) – Poster Sessions & Coffee Breaks in Foyer International

Tuesday 16 Oct	Wednesday 17 Oct	Thursday 18 Oct	Friday 19 Oct
Morning	8:30 – 11:50 ***Host Response and Immunity*** **Co-chairs:** **Katy Bosio, NIAID/NIH, USA** **Thomas Henry, INSERM, France** Topic overview, Katy Bosio Eight 20-minute talks from abstracts with coffee break	8:30 – 12:05 ***Pathogenesis and Cell Biology*** **Co-chairs:** **Lee-Ann Allen, Univ. of Iowa, USA** **Marina Šantić, University of Rijeka, Croatia** Topic overview, Marina Šantić Seven 20-minute talks from abstracts with coffee break	8:30 – 12:00 ***Human Infection and Treatment*** **Co-chairs:** **Max Maurin, Univ. Grenoble-Alpes, France** **Anders Sjöstedt, Umeå University, Sweden** Topic overview, Max Maurin Seven 25-minute + one 20 minute talk from abstracts with coffee break
	11:50 – 12:30 *Pitches for Poster Session #1* Chair: Petra Oyston, DTSL, United Kingdom	12:05 – 12:45 *Pitches for Poster Session #2* Chair: Jeannine Petersen, CDC, USA	
Lunch	12:30 – 13:15, Lunch at CMR 13:15 – 14:00, TULISOC meeting	12:45 – 13:45, Lunch at CMR	12:00 – 13:00, Lunch at CMR
Afternoon	14:00 – 16:30 ***Vaccines*** **Co-chairs:** **Terry Wu, Univ. of New Mexico, USA** **Karl Klose, UTSA, USA** **Helena Lindgren, University of Umeå, Sweden** Topic overview, Terry Wu Five 20-minute talks from abstracts + two tularemia product development discussions	13:45 – 16:00 ***Bacteriology and Gene Regulation*** **Co-chairs:** **Petra Oyston, DSTL, United Kingdom** **Simon Dove, Harvard University, USA** Topic overview, Simon Dove Five 25-minute talks from abstracts	13:00 – 16:00 ***Epidemiology and Ecology*** **Co-chairs:** **Kiersten Kugeler, Centers for Disease Control and Prevention, USA** **Raquel Escudero, Instituto de Salud Carlos III, Spain** Topic overview, Mats Forsman Six 20-minute talks from abstracts with coffee break
17:00 – 19:30 **Opening reception, McGill Faculty Club** *Keynote address:* Wayne Conlan, President, TULISOC	16:30 – 18:30 ***Poster Session #1, with refreshments***	16:00 – 18:00 ***Poster Session #2, with refreshments***	18:30 – 21:30 ***Tularemia 2018 Banquet:*** Awards, Future Meeting Plans and Closing Program *Vieux Port Steakhouse, Old Montreal*

K-1
Biothreat Agents: Weapons of Mass Distraction?

Wayne Conlan, National Research Council Canada, Ottawa, Ontario, Canada

Keynote address

Sticks and stones through to chemical and nuclear weapons have all been tested on the battlefield. Biological weapons (BW) are the exception to this, despite the known ability of microbes to wreak havoc upon human populations. Indeed, the USA and its allies as well as the former Soviet Union spent many billions of dollars developing stockpiles of BW agents and designing sophisticated delivery systems for deploying them on the battlefield. Nevertheless, save for crude attacks by the Japanese dropping plague-infected fleas on civilian populations during the Sino-Japanese war, BW agents have remained sheathed throughout two major world wars and many smaller ones during the last 100 years. Even in the face of recent chemical weapons use in the Middle East, countries therein who purportedly hold stockpiles of BW agents have signally failed to use them. My presentation will suggest causes for this restraint.

Session 1

Host Response and Immunity

S1-1
Francisella inside the cell: implications for innate immune recognition of intracellular bacteria

Klara Kubelkova[1], Milota Benuchova[1], Lenka Plzakova[2], Zuzana Krocova[3], Ales Macela[1]

[1]University of Defence, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [2]University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove, Czech Republic, [3]FMHS UoD, Hradec Kralove, Czech Republic

Innate immune response constitutes the first line of defense against bacterial infections. The dominant role in triggering and streamline of innate immune responses play the innate immune recognition process along with the intrinsic characteristics of the microorganism and the host. The time-structured epigenetic reprogramming of innate immune cells, creating the hierarchy of immune response functional modules, is critical for inducing and regulating the expression of the adaptive immune response. Recent evidence supports the concept that immune response to the bacteria is guided by the host cell (sub)type that realize primary interaction with a bacterium. We have used the *Francisella tularensis* experimental models to analyze the processes leading to innate immune recognition of *Francisellae*. Generally it is accepted that *Francisella* with its three TLR2 ligands is recognized by TLR2 associated with MYD88, which initiate the formation of mydosome and subsequently activation of NF-κB and pro-inflammatory cytokine production. However, the data from in vitro systems demonstrated that *Francisella* activates multiple signaling pathways, among them Akt, ERK, Rac/Cdc42, JNK/cJun, and/or p38 signaling modules in temporally separate phases; the phases that can mirror the interaction with individual cellular organelles as are intracellular membranes of endoplasmic reticulum and/or cellular mitochondrion. Along with the activation processes, *Francisella* interferes with activated signaling pathways by modulation of Akt signaling using SHIP activation or influence the assembly of TRAF6 and TRAF3 complexes that control the transcriptional responses of pattern recognition receptors by inhibiting K63-linked polyubiquitination. Based on data from different *Francisella* models, we have compiled the basic paradigms of the innate immune recognition concept that respects spatiotemporal character of the innate immune recognition. According to this concept, the innate immune recognition is the multistep process that is dependent on the modulation of epigenetic reprograming of innate immune cells by changing microenvironment in time.

This work was performed within the framework of Ministry of Defence of the Czech Republic - long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence and Specific research project SV/FVZ201707 from the Ministry of Education, Youth and Sport of the Czech Republic.

S1-2
CD200R limits the neutrophil niche for *Francisella tularensis* infection

Joshua Casulli[1], Mark Fife[2], Stephanie Houston[2], Stefano Rossi[1], Joshua Dow[1], Diane Williamson[3], Graeme Clark[3], Tracy Hussell[1], Riccardo D'Elia[4], Mark Travis[1]
[1]University of Manchester, Manchester, United Kingdom, [2]The University of Manchester, Manchester, United Kingdom, [3]DSTL, Salisbury, United Kingdom, [4]Defence Science and Technology Laboratory, Salisbury, United Kingdom

Control of pulmonary immunity is crucial in maintenance of tolerance and generation of protective immune responses against pathogens. Here, we identify an unexpected pathway that promotes host responses during bacterial infection of the lung. Expression of CD200 receptor (CD200R), a molecule previously associated with dampening immune responses in the lung, is required to promote effective clearance of the lethal intracellular bacterium *Francisella tularensis*. *F. tularensis* replicates in macrophages and neutrophils. A lack of CD200R expression increased infectious burden both *in vitro* and *in vivo*. *In vivo,* CD200R-deficiency led to enhanced bacterial burden in neutrophils, indicating CD200R normally limits the neutrophil niche for infection. Indeed, depletion of this neutrophil niche in CD200R$^{-/-}$ mice restored *F. tularensis* infection to levels seen in wild type mice. Mechanistically, CD200R-deficient neutrophils displayed significantly less reactive oxygen species production, suggesting that CD200R-mediated ROS production in neutrophils is necessary to limit the colonisation and proliferation of *F. tularensis*.

Overall, our data show for the first time that CD200R has been shown to promote an aspect of inflammation. Maintaining the antimicrobial properties of neutrophils via the CD200R pathway may represent a novel therapeutic approach for treating intracellular pathogens.

S1-3

Virulent but not attenuated *Francisella tularensis* resists IFN-γ dependent antimicrobial responses through manipulation of host macrophage mitochondrial function

Forrest Jessop[1], Robert Buntyn[1], Benji Schwarz[2], Catharine Bosio[2]
[1]NIAID, Hamilton, [2]Rocky Mountain Laboratories, Hamilton

IFN-γ signaling is critical for host defense against intracellular pathogens. Thus, the ability to evade and modulate anti-microbial effects of IFN-γ is an important feature of virulence. In the current study, we demonstrate that the highly pathogenic intracellular bacterium *Francisella tularensis* ssp. *tularensis* (Ftt), but not the attenuated live vaccine strain (LVS), resists IFN-γ mediated responses in Bone Marrow-derived Macrophages (BMdM) from C57Bl/6J mice early during infection. Canonical IFN-γ mediated host responses, including production of reactive oxygen and nitrogen species, only partially contributed to control of LVS infection. Using extracellular flux analysis, we found Ftt and LVS differentially altered mitochondrial bioenergetics, with Ftt enhancing mitochondrial function, while LVS infection caused mitochondrial dysfunction and increased glycolysis. Treatment with IFN-γ further enhanced mitochondrial dysfunction in LVS but not Ftt-infected BMdM through impaired electron transport chain complex II (CII) activity. Impaired CII activity was associated with increased levels of itaconate in LVS-infected BMdM following IFN-γ treatment. Bacterial replication was partially restored in itaconate deficient BMdM, supporting a role for itaconate in control of LVS infection. Anti-microbial effects of itaconate were not due to direct activity of the metabolite on LVS. Rather, our data suggest that the anti-microbial effect of itaconate stemmed from its ability to impair CII activity. These results present evidence for a non-canonical pathway by which IFN-γ controls intracellular infection through altered host metabolism.

S1-4
Novel insights into inflammasomes responses to *Francisella*

Thomas Henry[1], Brice Lagrange[2], Sacha Benaoudia[2], Pierre wallet[2], Anders Sjöstedt[3], Kyrylo Krasnykov
[1]CIRI, International Center for Infectiology Research, Inserm U1111, CNRS, UMR5308, Lyon, France, [2]CIRI, Lyon, France, [3]Umeå university, Umeå, Sweden

Inflammasomes are molecular complexes assembled in the cytosol in response to the detection of danger signal or pathogen-associated molecular patterns. Inflammasomes have been demonstrated to be key to fight *Francisella novicida* in mice while the data on human macrophages and *F. tularensis* species are limited. We have recently studied inflammasome activation in human primary macrophages demonstrating key differences with the mouse model. Indeed, our results demonstrate that the non-canonical caspase-4 inflammasome is the main inflammasome governing anti-*F. novicida* response in human macrophages. Furthermore, caspase-4 activation can be recapitulated by Francisella LPS transfection into human macrophage. Using this system and a genome-wide CRISPR-Cas9 screen, we identified a novel player in the Caspase-4-non-canonical inflammasome. Finally, we have characterized the role of the interferon-inducible Guanylate Binding Proteins (GBPs) as key antibacterial molecules targeting *F. novicida* and *F. tularensis* Live Vaccine Strain and promoting inflammasome activation. While highly virulent *F. tularensis* strains largely escape these responses, this work sets a framework to understand the complexity of inflammasome activation during *Francisella* infection.

S1-5
Francisella-immune T lymphocytes do not alter intramacrophage bacterial trafficking but instead limit replication and cell-to-cell spread of *F. tularensis* LVS

Kate Bradford[1], **Karen Elkins**[1]
[1]*CBER/FDA, Silver Spring*

Francisella tularensis is a highly infectious intracellular bacterium that causes tularemia by invading and replicating in host myeloid cells, especially macrophages. Immune T cells generated by vaccination or previous sublethal infection control murine *Francisella* infections in vivo, and immune T cells restrict the growth of *Francisella* bacteria within infected macrophages in vitro. Using the Live Vaccine Strain (LVS) of *F. tularensis* as a model, we studied how immune pressure from *Francisella*-immune T cells affects bacterial trafficking patterns within mouse macrophages. Specifically, we studied the possibility that T cells target bacteria for destruction by re-routing them into lysosomes and/or into autophagy pathways. Using mouse bone marrow-derived murine macrophages and LVS-immune mouse splenocytes, we adapted and optimized an established in vitro co-culture infection system to perform fixed and live-cell fluorescent confocal microscopy. Using this approach, we visualized the interactions between naïve or *Francisella*-immune lymphocytes and infected macrophages for the first time. We focused on late stages of infection, after phagosomal escape, through replication and the (presumed) death of macrophages. In agreement with previous studies, within 2 hours of infection we found LVS in EEA1$^+$, LAMP1$^+$ phagosomes and LVS then escaped to replicate in the cytoplasm. In contrast to previous research, while LVS continued to replicate over the next 24 hours, the majority of LVS was not sequestered into LAMP1$^+$ *Francisella*-containing vacuoles (FCVs) but remained cytosolic. Instead, we found a variety of phenotypes of masses of LVS bacteria that differed between infected macrophages. These varying patterns continued over the next 48-72 hours as LVS infection expanded and as macrophages proceeded to die. Live-cell imaging demonstrated that macrophage death due to LVS infection was a varied process and likely involves several pathways. Most importantly, LVS in macrophages co-cultured for up to 48 hours with either naïve or immune T cells did not co-localize with markers of the endosomal pathway (EEA1 and LAMP1), lysosomal pathway (Cathepsin D and Lysotracker), or autophagic pathways (LC3B), and remained cytosolic. Instead of re-routing, co-culture with *Francisella*-immune lymphocytes halted the replication of LVS within macrophages and inhibited the spread of LVS infection between macrophages. Thus, direct inhibition of intramacrophage bacterial growth is a major T cell immune mechanism.

S1-6

Temporal and functionally distinct roles for resident and circulating T cells during vaccine-mediated protection against tularemia

Lydia Roberts[1], Tara Wehrly[1], Robin Ireland[2], Deborah Crane[1], Catharine Bosio[2]
[1]Rocky Mountain Laboratories, NIAID, NIH, Hamilton, [2]Rocky Mountain Laboratories, Hamilton

The development and dynamics of the T cell response within the lung following vaccination and infection are complex. Within the pulmonary compartment, there are anatomically distinct pools of T cells that can generally be segregated into tissue resident and circulating populations. Each of these populations play discrete roles in combating infection. Specifically, during persistent (>60 days) or acute infections (<10 days) resident T cells are thought to be essential for rapid control of infection, whereas circulating T cells replenish this pool to maintain immunity. Vaccine-induced immunity against the highly virulent intracellular bacterium, *Francisella tularensis* (Ftt) is T cell dependent and clearance of the organism in immune animals takes 30-40 days. Therefore, immunity to Ftt serves as a model for understanding the roles of resident versus circulating T cells in an infection requiring longer periods of time for clearance. Following intranasal vaccination with the Live Vaccine Strain (LVS), we observed an increase in both resident and circulating effector $CD4^+$ T cells. However, while there was a significant increase in the number of tissue resident, poly-functional effector $CD4^+$ T cells, the circulating pool was only increased proportionality compared to naïve mice. To determine the contribution of resident or circulating T cells in the control of Ftt infection *in vivo*, we inhibited T cell trafficking from the circulation into the lung parenchyma using FTY720 among immune animals infected with virulent Ftt. In the first week of infection, mice treated with FTY720 had similar bacterial burdens as vehicle-treated mice suggesting resident T cells were sufficient for early control of Ftt replication. However, at later time points, FTY720-treated mice had significantly higher bacterial burdens, unconstrained inflammation, and did not survive Ftt infection. These results suggested a temporal requirement for resident and circulating T cells during infection that requires longer periods of time for clearance and is in direct contrast to other infections which originate in the lung. These data provide important insights into the roles of specific T cell populations that will be essential for design of novel effective vaccines against tularemia and potentially other agents of pulmonary infection.

S1-7
Macrophage selenoproteins restrict intracellular replication of *Francisella tularensis*

Rachel Markley[1], David Williamson[2], Bhuvana Katkere[1], Kalyan Dewan[1], Ashley Shay[1], Sarah Sumner[1], Bradley Carlson[3], K Sandeep Prabhu[1], **Girish Kirimanjeswara**[4]
[1]Penn State University, University Park, [2]Penn State University, University Park, PA, [3]NIH, Bethesda, [4]The Pennsylvania State University, University Park, PA

There has been a growing appreciation for the influence of dietary nutrients on the microbiome as well as host response to infectious disease. The micronutrient selenium (Se) is known to regulate immune functions via selenoproteins, a class of proteins that contain the 21st amino acid selenocysteine. However, the mechanisms by which selenoproteins regulate immune functions during an acute infection are not clear. Therefore, we investigated the role of macrophage (Mac) selenoproteins during an acute bacterial infection. *Francisella tularensis (Ft.),* the causative agent of tularemia, is a gram-negative intracellular bacterium. Since *Ft.* infects and replicates primarily in Macs, we measured the bacterial growth in Macs derived from Trsp^{M} mice that are unable to synthesize selenoproteins. Trsp^{M} Macs had uncontrolled growth of *Ft.* compared to wild-type (WT) Macs. Moreover, Trsp^{M} mice were more susceptible to *Ft*. infection and harbored significantly higher levels of bacteria in their livers and spleens as compared to WT mice, which suggests that Mac selenoproteins are essential for restricting bacterial growth and promoting host survival. Since *Ft.* growth is closely associated with autophagy, we investigated if the Mac selenoproteins had an effect on autophagy. In fact, our data indicate that selenoproteins inhibit autophagy, which may limit the availability of nutrients to the bacteria. Our studies demonstrate that nutrients affect the outcome of an infection by influencing host immune response and reveal potential novel targets for antibacterial therapies.

S1-8
Evaluation of the therapeutic potential of an anti-inflammatory manno-dendrimer in a mouse model of *Francisella tularensis* infection

Camille Robert[1], Sonia Touron[1], Alain Charbit[2], Jacques Prandi[1], Alain Vercellone[1], **Jérôme Nigou**[1]
[1]Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, Toulouse, France, [2]INSERM, Paris Cedex 14, France

Pulmonary tularemia is characterized by an initially delayed immune response followed by a hypercytokinemia that results in a massive recruitment of immune cells in infected tissues. Accumulation of inflammatory cells, most particularly neutrophils, leads to severe tissue damages and plays an important role in the pathology and mortality of this disease. Tularemia treatment is based on antibiotics, which are often administered too late because of a difficult diagnosis and are therefore less efficient. Thus, new therapeutic strategies are needed to replace or complement antibiotherapy. In this context, we aimed to investigate whether reducing the excessive inflammatory response caused by *Francisella tularensis* could be beneficial for the infected host and be used as an adjunctive therapy. Here, we evaluated the therapeutic potential of a new synthetic anti-inflammatory compound, called mannodendrimer 3T (M3T), designed in our group to bind the C-type lectin DC-SIGN and to mimic a natural anti-inflammatory lipoglycan produced by *Mycobacterium tuberculosis*. M3T was previously shown to reduce cytokine production and neutrophil recruitment in a mouse model of acute lung inflammation induced by LPS (Blattes *et al*., PNAS, 2013). Interestingly, we demonstrate that M3T is able to inhibit the production of pro-inflammatory cytokines and activation of NF-κB, a central transcription factor in inflammation, in macrophages infected with *F. tularensis* subsp. *novicida.* Moreover, *in vivo,* administration of M3T in adjunction with a suboptimal dose of gentamicin increases the survival rate of mice infected with *F. novicida* as compared to antibiotic alone. In addition, preliminary experiments indicate that M3T reduces inflammatory pathology and tissue damages in lungs and liver. Altogether, our data suggest that an anti-inflammatory adjunctive therapy might be of interest for the treatment of pulmonary tularemia.

Session 2

Vaccines

S2-1
Small particle aerosols of *Francisella tularensis*; characterization and optimization to support pivotal animal efficacy studies

Doug Reed[1], Jennifer Bowling[1], Katherine Willett - O'Malley[2]
[1]University of Pittsburgh, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA-PENNSYLVANIA

Evaluating vaccines and therapeutics for efficacy in animal models under the FDA's Anima Rule requires development of a well-characterized and reproducible method for challenging animals. For bioterrorism threats like *Francisella tularensis* (*Ft*), aerosol dissemination is the likeliest means of a potential attack. Because the FDA has stated that intranasal and intratracheal inoculations do not sufficiently mimic inhalation, pivotal animal efficacy studies must involve delivery of the challenge agent via small particle aerosol. Aerosol exposures can be technically challenging and reproducibility, especially in terms of inhaled dose, can be quite difficult. We sought to characterize and optimize aerosolization of tularemia to reduce variability between exposures. Spray Factor (SF), the ratio of the aerosol concentration to the nebulizer concentration, is used to assess aerosol performance. Relative humidity and chamber volume had significant impacts on the SF for *Ft*; relative humidity above 65% improved SF by more than 1 log10. The broth media used to culture Ft for challenge also impacted SF. BHI-grown *Ft* has the highest SF, typically 1 log10 or better than MHb- or CCDM-grown *Ft*. This means that 10-fold less BHI-grown *Ft* compared to MHb- or CCDM-grown *Ft* is needed to achieve the same target dose in an animal. BHI-grown Ft aerosols also have little run-to-run and day-to-day variability, which will improve reproducibility and precision in dosing. Slight differences in the OD600 of the challenge material resulted in a 5-fold increase in the actual dose compared to the target dose in a rabbit vaccine study. Diluting the challenge material to an OD600 within a narrow range (0.05 – 0.3) that fit a linear regression model produced the desired starting concentration. This greatly improved accuracy in achieving the desired target dose. At a target dose of 1,000 CFU the average delivered presented dose was 1,025 CFU with a coefficient of variation of 0.21. Differences in individual animal minute volume during the 10-minute exposure largely account for the remaining variation seen. We are currently evaluating whether we can eliminate differences in respiratory function between individual animals using volume-based instead of duration-based aerosols, which would further improve accuracy and precision in Ft dosing. This system is sufficiently robust and reproducible to meet the needs for pivotal efficacy studies.

S2-2

Evaluation of the *Ft-ΔClpB* vaccine using working immune correlates predicts protection against *Francisella* challenge

Roberto De Pascalis[1], Blake Frey[1], Helen Brook[1], Wayne Conlan[2], Anders Sjöstedt[3], Herbert Carl Gelhaus[4], Karen Elkins[1]
[1]CBER/FDA, Silver Spring, [2]National Research Council Canada, Ottawa, Ontario, Canada, [3]Umeå university, Umeå, Sweden, [4]MRIGlobal, Kansas City, MO

The extent of protection provided by the Live Vaccine Strain (LVS) against challenge with the virulent Type A *Francisella tularensis* (*Ft*) is not clear. Better prophylactic vaccines are needed, as well as better methods to test their efficacy due to the difficulties in conducting field trials. We have described a strategy that predicted the degree of vaccine-induced protection in LVS-vaccinated mice and rats given either parenteral or respiratory *Francisella* challenges. This approach consists of *in vitro* stimulation of *Ft* LVS-immune cells and analyses of their functions and gene expression. Here, we used this approach to investigate correlates of protection for a vaccine derived from the Type A *Ft* strain SchuS4, *Ft*-Δ*clpB* vaccine. In mouse studies, *Ft*-Δ*clpB* appeared to provide better protection than LVS against aerosol challenge with fully virulent *Ft*. C57BL/6 mice and Fisher 344 rats were vaccinated with *Ft*-Δ*clpB* as well as LVS-derived vaccines, and animals were subsequently challenged with lethal doses of LVS or *Ft* SchuS4. In parallel, *in vitro* functions of naïve and immune PBLs and splenocytes were evaluated and compared to the *in vivo* survival patterns. In general, leucocytes from *Ft*-Δ*clpB* vaccinated animals controlled intramacrophage *Francisella* LVS growth in *in vitro* co-culture assays and produced nitric oxide to levels that were comparable to or exceeded those of leukocytes from LVS-vaccinated rodents. Leukocytes recovered from co-cultures were evaluated for relative gene expression using a panel of genes previously identified in *Francisella* mouse and rat studies as well as in mouse studies of another intracellular bacterium, *Mycobacterium tuberculosis*. Up-regulation of several genes in lymphocytes from *Ft*-Δ*clpB*-vaccinated rodents was higher than levels quantitated in LVS-derived leukocytes, suggesting that different qualitative and/or quantitative immune responses may be responsible for the improved protection by this vaccine. These data not only demonstrate that this correlate strategy is suitable for screening Type A-based vaccines, but also confirm the promising characteristics of the *Ft*-Δ*clpB* vaccine and support advancing this vaccine candidate to further studies in non-human primates.

S2-3
A protective subunit vaccine against tularaemia: delivery of antigens by glucan particles induces protection in rats

Petra Oyston[1], Adam Whelan[2], Helen Flick-Smith[2], Stuart Levitz[3], Gary Ostroff[3]
[1]Dstl, Salisbury, United Kingdom, [2]Dstl, Porton Down, United Kingdom, [3]University of Massachusetts, Worcester

Protection against *Francisella tularensis* requires both humoral and cellular immune memory responses. Subunit approaches have failed to induce protection despite testing of many candidates and have been hampered by a lack of understanding as to which antigens are immunoprotective. LPS has been shown to induce partial protection in mice, but this protection requires supplementing by other antigens to induce protection against the most highly virulent strains. We undertook a preliminary in silico analysis to identify candidate protein antigens. These antigens were then recombinantly expressed and encapsulated into glucan particles (GPs), purified *Saccharomyces cerevisiae* cell walls composed primarily of β-1,3-glucans. Immunological analysis in mice allowed down-selection to proteins which induced both antibody and T cell memory responses. Rats were immunised with the most promising proteins and LPS delivered in glucan particles, and we were able to induce protection in rats against a robust aerosol challenge. We thus identified a protein capable of supplementing protection induced by LPS in a tularaemia vaccine, demonstrating the adjuvanticity provided by the GP vaccine platform. This paves the way for developing an effective, safe subunit vaccine for the prevention of inhalational tularemia.

Funding was provided by the Defence Reduction Agency under contract HDTRA1-12-D-0003-0008
© Crown copyright (2018), Dstl. This material is licensed under the terms of the Open Government Licence except where otherwise stated. To view this licence, visit http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3 or write to the Information Policy Team, The National Archives, Kew, London TW9 4DU, or email: psi@nationalarchives.gsi.gov.uk”

S2-4
Evaluation of a plug and play approach for the development of multi-antigen subunit vaccines

Chandra Bakshi[1], Ahd Mansour[2], Paul Arnaboldi[2], Alison McCormick[3], Meenakshi Malik[4]
[1]New York Medical College, Valhalla, NY, [2]New York Medical College, Valhalla, [3]Touro University, Vallejo, [4]Albany College of Pharmacy and Health Sciences, Albany, New York

Francisella tularensis (*Ft*) and *Yersinia pestis (Yp)* are the causative agents of tularemia and plague respectively. Both are highly lethal human pathogens, with strong historical evidence supporting concerns about their potential use as agents of biowarfare/bioterror. Currently, no FDA approved vaccines are available in the US for prevention for plague or tularemia. In this study we utilized Tobacco Mosaic Virus (TMV) as a novel vaccine delivery platform to develop an effective protein subunit vaccine for protection from lethal pneumonic plague and tularemia. In this approach, individual protein antigens were conjugated to the surface of TMV and then multiple TMV-protein conjugates were mixed and optimized into a single vaccine formulation and delivered intranasally (i.n.). We demonstrate that candidate plague and tularemia vaccines; consisting of antigens from *Yp* or *Ft* conjugated to TMV could be administered i.n. to mice without adverse reactions; induce potent adaptive immune responses to *Yp* and *Ft* antigens; and provide 100% protection in immunized mice against lethal pneumonic plague and tularemia. Furthermore, a single multi-antigen TMV vaccine formulation consisting of *Ft* and *Yp* antigens is capable of protecting against lethal sequential challenge with both plague and tularemia. Collectively, this study provides a concrete proof-of-concept that mucosal delivery of multiple antigenic proteins linked to TMV induces complete protection against both pneumonic plague and tularemia, and that effective single vaccines against multiple biothreat agents can be generated using the novel “plug-n-play” aspect of the TMV delivery platform.

S2-5
Engineering OAg^{FTT} expression in *F. novicida*

Xhavit Zogaj[1], Aanuoluwa Adelani[2], Jesse Nguyen[3], Ping Chu[2], Karl Klose[4]
[1]The University of Texas at San Antonio, San Antonio, TX, [2]Univeristy of Texas at San Antonio, San Antonio, [3]Univeristy of Texas at San Antonio, San Antonio, TX, [4]University of Texas San Antonio, San Antonio, TX

Francisella tularensis subsp. *tularensis* (*Ftt*) is a highly virulent bacterium that causes the disease tularemia. Ftt can cause a fatal infection in humans when the bacteria are inhaled into the lung, therefore Ftt has been classified as a category A biothreat agent. *F. novicida* (Fn) is a closely-related species that is avirulent in healthy humans but causes a fatal disease in mice. We are developing Fn as a live vaccine strain to protect against Ftt infection. We have already shown that Fn with a mutation in *iglD*, the T6SS gene required for phagosome escape and intramacrophage replication (Fn-*iglD*), protects vaccinated animals against pulmonary challenge with Ftt using two different animal models, the Fischer 344 rat and the cynomolgus macaque. To enhance the efficacy of Fn-*iglD*, we engineered it to express the LPS O-Antigen from Ftt (OAg^{FTT}). This process involved the removal of the entire OAg cluster consisting of 12 genes from Fn-iglD, followed by the stepwise replacement of the OAg cluster consisting of 15 genes from the Ft subsp. holarctica LVS strain, which expresses OAg^{FTT}. The resultant strain, KKF768 (Fn-iglD OAg^{FTT}), expresses OAg^{FTT} attached to its LPS. Importantly, vaccination of both mice and rats with Fn-iglD OAg^{FTT} via the pulmonary route induces serum antibodies against OAg^{FTT}. Moreover, vaccination of Fischer 344 rats with Fn-iglD OAg^{FTT} provided protection against lethal pulmonary challenge with Ftt Schu S4, demonstrating that Fn-iglD OAg^{FTT} is a good candidate for a tularemia vaccine.

S2-6
Options in the United States for licensing new biological products for rare infectious diseases

Sherry Kurtz[1], Karen Elkins[1], Roberto De Pascalis[1]
[1]*CBER/FDA, Silver Spring*

Clinical trials to evaluate new drugs, vaccines, and therapeutics are difficult to design for sporadic diseases like tularemia. In the U.S., evaluation of efficacy based on animal studies is now an option using the “Animal Rule.” A number of criteria must be met in order to invoke this option, and a variety of well-characterized tools need to be available. These issues will be discussed in the context of development of new products to combat tularemia.

S2-7
Making the Cut: Considerations for Moving Candidates from Discovery to Advanced Development

Robert House[1], **Ronald Cobb**[2]
[1]Ology Bioservices, Frederick, Maryland, [2]Ology Bio, Frederick

The enormous costs of advanced development for vaccines and therapeutics, as well as the ethical and humanitarian mandate to develop these products as quickly (and safely) as possible, requires that companies exercise a degree of discrimination when choosing these candidates. This presentation will describe a number of such considerations including estimated probability of success of the eventual product, likelihood of sustained support, regulatory pathways, return on investment and risk in all its many incarnations.

Poster Session #1

P1-01
Identification of the causative *Francisella* strain of an uncommon tularemia outbreak in fall 2016, Germany

Kristin Koeppen, Daniela Jacob, Roland Grunow, Klaus Heuner
[1]*Robert Koch Institute, Berlin, Germany*

Introduction: *Francisella tularensis* (*Ft*.) is an intracellular pathogen causing tularemia in a variety of hosts including humans and rodents. Various transmission and infection routes have been described causing different clinical manifestation like massive swollen lymph nodes and pneumonia. So fare, all reported cases of tularemia in Germany are caused by the *Ft.* subspecies *holarctica* (*Fth*.) which is distributed in the whole northern hemisphere. In fall 2016, six participants of a grape harvest in Germany developed tularemia after drinking freshly pressed grape must. Since this case is unique to our knowledge, we wanted to identify the causative *Ft*. strain and postulate the transmission scenario. **Material and Methods:** Chromosomal DNA was extracted from grape must products and used in a PCR (RD1-PCR) detecting specifically *Ft*. DNA. Next generation sequencing (NGS) was performed to identify the outbreak *Ft.* strain for additional phylogenetic classification. Also, extracted must DNA samples were used in a vertebrate-specific cytochrome b PCR. The obtained PCR product was cloned into the pGEM-T Easy vector for further analyses.

Results: *Fth.* DNA was detected within grape must samples using the RD1-PCR. Since quantitative PCR indicated a relatively high amount of genome equivalents per ml in must samples, they were successfully sequenced by NGS. Bioinformatic analyses of NGS data revealed that the *Fth*. 'must' strain belonged to the phylogenetic clade B.12/B.34 (representing Erythromycin-resistant Biovar II). In addition, NGS data were collected and analysed from DNA samples extracted from a tularemia patient of the outbreak. These data confirmed that the patient had been infected with the same *Fth*. B.12/B.34 genotype. Moreover, mitochondrial DNA of *Apodemus sylvaticus* was found in must samples but not in other case unrelated samples using the vertebrate-specific cytochrome b PCR.

Conclusion: Here, we identified and characterized the causative *Ft*. strain of the uncommon tularemia outbreak in fall 2016. Due to the fact that DNA of *A. sylvaticus* was found in must samples, we concluded *A. sylvaticus* infected with *Fth*. as the potential contamination source.

P1-02
Secretion of outer membrane vesicles in *F. tularensis* and a set of mutant strains with disrupted surface structures

Jan Bavlovič[1], Jana Klimentova[1], Jiri Stulik[2]
[1]University of Defense, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [2]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

Bacterial outer membrane vesicles (OMVs) are particles of spherical shape of 20-200 nm, which are formed by the bulging and separation of a part of the bacterial outer membrane. OMVs are produced by all gram-negative bacteria and are considered to be one of the main ways of secretion that serves for the interaction with both other bacterial cells and eukaryotic cells. Excretion of the vesicles is generally considered as an adaptive response to stress and OMVs also may have several tasks during the infection, especially in interaction with human immune system. In a recent study by McCaig et al. (2013), it has been shown that *F. novicida* produces, in addition to classical OMVs, also membrane vesicles of unusual tubular shape. Former experiments performed at our workplace using electron microscopy have shown the secretion of similar tubular vesicles also in *F. tularensis* subsp. *holarctica* (strains FSC200, a clinical isolate) as well as the highly virulent subsp. *tularensis* (SchuS4 strain). Proteomic analysis of isolated OMVs from these strains revealed a large number of known virulence factors as well as immunostimulatory proteins. As such they represent a very promising candidate for subunit vaccine research.

As the biogenesis of OMV occurs on the bacterial surface we focused on the vesiculation in a group of mutants with disrupted surface structures like type IV pili, lipopolysaccharide or the polysaccharide capsule. Several mutants with a known attenuation in the intracellular lifestyle were also selected to evaluate whether OMV are involved in any phase of the pathogenicity. The vesiculation rate of these mutants was evaluated by fluorescence labelling and compared to that of the wild type strain in order to find possible high- or low-vesiculating variants. The OMV morphology of selected strains was further evaluated by electron microscopy. The results suggest that the integrity of LPS and the polysaccharide capsule affect OMV size, shape and abundance.

The study was financially supported by GAČR (17-04010S), Ministry of Defense of the CR (Long-term organization development plan Medical Aspects of Weapons of Mass Destruction) and Ministry of Education, Youth and Sports (SV/FVZ201804).

P1-03
Influence of sex on protection conferred by vaccination with attenuated strains of *Francisella tularensis* in the rabbit model

Jennifer Bowling[1], Elizabeth Stinson[2], Katherine Willett - O'Malley[3], Karsten Hazlett[4], Eileen Barry[5], Doug Reed[1]
[1]University of Pittsburgh, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, [3]University of Pittsburgh, Pittsburgh, PA- PENNSYLVANIA, [4]Albany Medical College, Albany, [5]University of Maryland at Baltimore, Baltimore, MD

Francisella tularensis (Ft), the causative agent of tularemia, has a 30% fatality rate in humans. This bacterium can be spread through arthropods, direct contact, ingestion, and inhalation, resulting in different forms of disease, of which the pneumonic form is the most severe. Ft is considered a category A select agent by the CDC due to its low infectious dose, ease of dissemination, and potential for severe disease. In the rabbit model, we have previously shown that recombinant derivatives of virulent Ft strain SCHU S4 are attenuated and can serve as vaccines, protecting New Zealand White rabbits against aerosol challenge with SCHU S4. Protection is dependent on vaccination route, number of doses, and vaccine strain used. A prime-boost approach with two aerosol doses of S4ΔaroD delivered two weeks apart resulted in 65% survival of female rabbits aerosol challenged with 200 LD_{50} of SCHU S4 (11 of 17 female rabbits). Based on a recent report showing better protection in female mice compared to male mice, we evaluated whether sex could influence the protection seen in rabbits. When male rabbits were vaccinated by prime-boost with aerosol S4ΔaroD, 11 of 13 (85%) survived aerosol challenge with 200 LD_{50} of SCHU S4. Differences were also noted in the response to vaccination, with female rabbits losing more weight after the prime vaccination than male rabbits. Although antibody titers were high in rabbits from both sexes that were vaccinated, plasma IgG on day 28 post-vaccination was higher in female than male rabbits. Vaccinated male rabbits that survived challenge had higher levels of granulocytes in the blood on day 4 post-challenge; this increase was not seen in surviving female rabbits. These findings suggest that the observed difference in survival of vaccinated male and female rabbits may be a result of differences between the sexes in the cellular and humoral immune responses.

P1-04

Immunostimulatory Activity of Dillapiole Isolated from Fennel Root

Elliot Collins[1], Deanna Schmitt[2], Joseph Horzempa[3]
[1]West Liberty University, West Liberty, WV, [2]West Liberty University, West Liberty, West Virginia, [3]West Liberty University, West Liberty, WV, WV

*Francisella tularensis*is a highly infectious bacterium that causes the disease, tularemia. The Centers for Disease Control and Prevention classified *F. tularensis*as a category A bioterror agent due to its virulence and ease of aerosolization. The intentional release of a resistant strain of *F. tularensis*could be devastating. Consequently, there is a need for novel treatments effective against *F. tularensis*infections. We previously tested a cataloged natural compound library for inhibition growth of a fluorescent *F. tularensis*strain (LVS/pTC3D) during infection of THP-1 monocyte cells. From this analysis, we identified nine extracts that only limited bacterial replication in the presence of these host cells. One of the most promising extracts for the inhibition of *F. tularensis*during infection was from Fennel. Using bioassay guided fractionation, the Fennel extract was separated until a pure compound was isolated and identified using NMR and mass spectrometry. The active compound was determined to be dillapiole, an allylbenzene that has previously been extracted from several plant species. We sought to determine whether dillapiole would be effective at diminishing disease caused by other infectious bacteria, such as the opportunistic pathogen, *Acinetobacter baumannii*. Therefore, wax worms infected with *A. baumannii*were treated with dillapiole or were mock treated. Wax worm larvae treated with dillapiole exhibited significant survival compared to the control-treated insects. We are currently investigating the mechanism of activity of dillapiole, a compound that could have potential as a novel immunostimulatory therapeutic.

P1-05
The role of AMPK in *Francisella tularensis* intracellular growth

---

Sedelia Dominguez[1], Shaun Steele[2], Thomas Kawula[3]
[1]Washington State University, Pullman, WA, [2]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, [3]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA

Francisella tularensis is a highly virulent intracellular pathogen that rapidly proliferates in the cytoplasm of infected cells. To achieve high levels of growth, *F. tularensis* must harvest and utilize essential nutrients from within the cell. Since most essential nutrients are not readily available in the host cell cytosol, *F. tularensis* must alter host metabolism to generate usable carbon and energy substrates. The strategies of manipulation of host metabolic processes for *F. tularensis* are not fully known. We found that a master metabolic regulator, AMP-activated protein kinase (AMPK), was activated in *Francisella* infected cells, and that *Francisella* intracellular growth was significantly impaired in AMPK knockout cell lines. AMPK activation impacts many host cell processes, including but not limited to increased glucose import and degradation of internal complex carbon and energy storage molecules. To examine the role of AMPK and AMPK activation in *Francisella* intracellular growth, we created a *Francisella* transposon insertion library composed of mapped insertion mutants that are defective for intracellular growth. We screened this library for intracellular growth defective mutants that were rescued by addition of the AMPK activating drug, AICAR. AICAR treatment rescued the growth of one mutant, FTL_1648 TN, to half that of wild type. Both wild type and FTL_1648 TN mutant exhibited diminished growth that was not rescued with treatment of AICAR in AMPK knockout cells. Therefore, AMPK signaling is an important factor for intracellular growth. Our data identifies a potential bacterial effector that regulates the activation of AMPK. Additionally, our data identifies a host metabolic pathway employed during *F. tularensis* infection necessary for nutrient acquisition.

P1-06
Mechanism of the host-mediated process of *Francisella tularensis* cell-cell transfer

---

Amanda Foreman[1], Shaun Steele[1], Thomas Kawula[2]
[1]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, [2]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA

Francisella tularensis has been shown to transfer directly from infected macrophages to uninfected macrophages through a host-mediated process. This direct cell-cell transfer may contribute to *F. tularensis* dissemination during infection. Bacterial cell-cell transfer is not well understood, and therefore our goal is to elucidate the mechanism of this process. Lipopolysaccharide (LPS) treatment resulted in increased transfer from infected to uninfected murine bone marrow-derived macrophages (BMDMs). LPS typically stimulates an inflammatory response by binding Toll-like receptor 4 (TLR4) and signaling through myeloid differentiation adaptor protein, MyD88. However, we found that enhanced cell-cell transfer by LPS treatment was MyD88 independent. TLR4 can also activate spleen tyrosine kinase (SYK), we therefore investigated the potential role of SYK in regulating bacterial cell-cell transfer. We found that the SYK inhibitors R406, BAY 61-3606, and piceatannol, decreased Schu S4 transfer from infected to uninfected BMDMs. SYK is involved in numerous cell signaling pathways, including those associated with cell-cell adhesions, phagocytosis, and cell motility. We therefore, screened a number of inhibitors of SYK-dependent pathways to determine which were responsible for impacting bacterial cell-cell transfer. Preliminary data suggests that inhibition of Rac and Vav1 proteins leads to a defect in bacterial cell-cell transfer. Rac and Vav1 are involved in regulating cell-cell adhesion, suggesting that Rac and Vav1 regulated adhesion molecules may significantly contribute to bacterial transfer. Understanding the mechanism of cell-cell transfer of *F. tularensis*could lead to novel therapeutics that could block the rapid dissemination.

P1-07
The role of Guanylate-binding proteins (GBPs) during intracellular infection with *Francisella tularensis*

Nasibeh Mohammadi[1], Helena Lindgren, Thomas Henry[2], Wolfgang Schröder[1], Anders Sjöstedt
[1]Umeå University, Umeå, Sweden, [2]CIRI, International Center for Infectiology Research, Inserm U1111, CNRS, UMR5308, Lyon, France

Francisella tularensis (*F. tularensis*) is a facultative intracellular Gram-negative bacterium and the causative agent of the zoonotic disease tularemia. IFN-γ is crucial to control infection with this bacterium. However, it is unclear how the critical role of IFN-γ for control of cytosolically localized bacteria is executed, but recent studies on *Francisella novicida* (*F. novicida*), a bacterium closely related to *F. tularensis*, showed that GBP2 and GBP5, two major proteins from the GBP family, targeted cytosolic *F. novicida and* promoted their lysis. The bacterial genomic DNA released into host cytosol was proposed to activate the AIM2 inflammasome. The role of GBPs for control of *F. tularensis* infection is much less studied. In view of the lack of understanding regarding how the antibacterial mechanisms are mediated by GPBs and the lack of knowledge regarding their role for control of other *Francisella* strains, both of these questions are critical for a comprehensive understanding of the role of GBPs. In this study we specifically assessed how GBP2 affects intracellular growth of *F. tularensis* LVS or SCHU S4 and *F. novicida*, in both mouse and human cells, and if the AIM2 inflammasome is required for the GBP2 antibacterial activities in *F. tularensis* strains. Preliminary data on mouse macrophage cells indicates that GBP2 are important to restrict intracellular growth of the *F. tularensis* LVS strain and *F. novicida*, however, the SCHU S4 strain is not affected by the GBP2-mediated control.

P1-08
Characterization of MFS Transporters FptG and FptB in Type A *Francisella tularensis*

Eileen Barry[1], Phillip Balzano[2]
[1]*University of Maryland at Baltimore, Baltimore, MD,* [2]*Universiyt of Maryland School of Medicine, Baltimore*

Francisella tularensis (Ft) is a facultative intracellular coccobacillus, and is the causative agent of tularemia. Due to past weaponization, a low aerosol infectious dose, and a high mortality rate for untreated disease, *Ft* is a Tier 1 select agent and a high priority for countermeasure development. Towards the goal of developing a safe and efficacious live attenuated vaccine, we evaluated two members of the major facilitator superfamily (MFS) transporters, *fptG* and *fptB,* as targets for deletion in the Type A strain Schu S4. The intracellular replication and escape kinetics of these mutants was altered in THP-1 human macrophage-like cells compared to wild type (WT) *Ft*. WT *Ft* began to escape and cause cell death (measured as LDH release) between 15 and 21 hours post infection (hpi)**.** SchuS4Δ*fptB* displayed an intracellular replication defect and began escaping 12 hours later than WT, with a corresponding delay in LDH release. The replication defect was repaired by complementation or with the exogenous addition of isoleucine, the substrate of the FptB transporter. SchuS4Δ*fptG* was delayed in escape by ~6 hours compared to wild type. While all strains induced robust levels of Il-1β release from infected cells, secretion kinetics mirrored those of LDH release. The quantity and timing of TNFα release was also significantly altered in cells infected with both mutants compared to WT. Despite these cellular effects, SchuS4Δ*fptG* was minimally attenuated in mice with an LD_{50} of 20 CFU (compared to WT LD_{50} ~ 1 CFU). In contrast, SchuS4Δ*fptB* was significantly attenuated compared to WT with an LD_{50} >10^6 CFU. A single intranasal dose of SchuS4Δ*fptB* was able to protect 35.7% of C57BL/6 mice from virulent WT challenge. These studies have confirmed the importance of two MFS transporters in the pathogenesis of the facultatively intracellular Type A pathogen *Ft* and have provided further insight into mechanisms of virulence. Further investigation is ongoing to assess the usefulness of these transporters as targets for the development of safe and efficacious live attenuated vaccines

P1-09
A putative low molecular mass penicillin binding protein is essential for virulence in *Francisella tularensis*

Joel Bozue[1], Sherry Mou[2], Sharon Daye[1], Patricia Worsham[1], Todd Kijek[3]
[1]USAMRIID, Ft Detrick, [2]USAMRIID, Ft. Detrick, [3]USAMRIID, Ft. Detrick, MD

Low molecular mass penicillin binding proteins (LMM PBP) are bacterial enzymes involved in the final steps of peptidoglycan biosynthesis. In *Escherichia coli,* most LMM PBP exhibit DD-carboxypeptidase activity and are not essential for growth in routine laboratory media. In addition, their role in virulence remains largely unknown. *Francisella tularensis* loci *FTT_1029* is putatively annotated as *dacD* and encodes for a LMM PMP with homology to PBP6b of *E. coli*. Unlike PBP6b in *E. coli*, disruption of this loci in the fully virulent Schu S4 strain resulted in a mutant strain that could not grow in Chamberlain's Defined Medium (CDM) and exhibited severe morphological defects as seen in electron microscopy. Further characterization demonstrated that these growth and structural defects related to the pH of the medium and could be partially restored when the pH was increased for the growth medium or fully restored in a genetically complemented strain. In addition, the *dacD* mutant was shown to be highly susceptible to various detergents, such as SDS and Tween-20. Resistance to wild-type levels could be restored when a functional gene was provided to the mutant. Infection of murine macrophage-like cells showed that the Schu S4 *dacD* mutant was capable of limited intracellular replication. However, this mutant was highly attenuated for BALB/c mice following intranasal challenge (LD_{50} = 600 CFU) as compared to mice challenged with the parent (LD_{50} = 1 CFU) or complemented strain (LD_{50} = 1 CFU). Additionally, mice that survived infection with the Schu *dacD* mutant showed significant protection against subsequent challenge with the parent strain. Collectively, these results indicate that FTT_1029 is essential for *F. tularensis* to grow in low pH environments and virulence. These results also suggest that a PBP mutant could serve as the basis of a novel, live attenuated vaccine strain.

P1-10
Investigating the role of cell morphology in virulence and persistence of the intracellular pathogen, *Francisella tularensis*

Stuart Cantlay[1], Joseph Horzempa[2]
[1]West Liberty University, West Liberty, [2]West Liberty University, West Liberty, WV, WV

Many species of bacteria, under conditions of stress or nutrient limitation, enter a state of dormancy referred to as viable but non-culturable (VBNC). Microorganisms that have entered the VBNC state persist in the environment, are difficult to detect and identify by many standard laboratory methods and can be altered in their susceptibility to antibiotics. Entry into the VBNC state is often accompanied by morphological changes; however, the mechanisms underlying this are poorly understood. Recent findings relating to VBNC in *Escherichia coli* suggest that there may be evolutionarily conserved mechanisms underlying these changes and that they can have broad implications for persistence and resuscitation of pathogenic bacteria. *F. tularensis* enters a VBNC state rapidly in laboratory culture but little is known about how this occurs or the biological significance of this transition. Microscopic observations of *F. tularensis* LVS and *F. novicida* reveal that cultures undergo morphological differentiation coincident with entry into the VBNC state. Unlike many of the other organisms for which VBNC has been studied, *Francisella* transitions rapidly and spontaneously to this state, and therefore has the potential to be an excellent model organism for the study of this phenomenon. In order to characterize the morphological changes of *F. tularensis*, fluorescence microscopy using membrane (FM4-64), DNA (Hoechst 33342) and live/dead (SYTO 9/propidium iodide) staining has been used to investigate the cell biology of *F. tularensis* LVS during the transition into the VBNC state. Preliminary data shows that VBNC *F. tularensis* LVS are able to invade and stimulate pro-inflammatory immune responses in RAW 264.7 mouse macrophages *in vitro* suggesting that these cells, whilst unculturable in the laboratory are not biologically inert. Finally, a strategy to investigate the homologs of key determinants of bacterial cell shape, *ftsZ, mreB* and *rodA* in relation to morphological changes during transition into the VBNC state will be outlined and preliminary results presented.

P1-11
Therapeutic outcome in tularemia patients since the introduction of fluoroquinolones as first-line drugs

Yvan Caspar[1], Max Maurin[2]
[1]Grenoble Alpes University Hospital, Grenoble, France, [2]CHU Grenoble Alpes, Université Grenoble Alpes, Grenoble, France

Treatment of tularemia remains challenging. Therapeutic failure may occur in 20-50% of cases, especially in case of delayed diagnosis and treatment, and despite no *F. tularensis* strains resistant to currently used antibiotics have been reported so far. According to the 2007 WHO guidelines, treatment of tularemia mainly relies on three antibiotic classes: the aminoglycosides, the fluoroquinolones and the tetracyclines. Treatment of severe tularemia forms in hospitalized adults consists in 10 days of intravenous gentamicin, while the alternative streptomycin is no longer available in many countries. In milder form of the disease parenteral therapy can be switched to oral. Ciprofloxacin for 10-14 days or doxycycline for 14-21 days are recommended. First use of fluoroquinolones was introduced in 1989 in Finland resulting in the complete cure of 5 volunteers suffering from acute tularemia (Syrjälä *et al*, 1991). Although still not FDA approved for this disease, many tularemic patients have been treated with fluoroquinolones. However, as tularemia is a rare disease, no large randomized studies comparing therapeutic outcome in tularemia cases treated either with aminoglycosides, fluoroquinolones or tetracyclines are available. We reviewed data of the literature available for *F. tularensis* antibiotic susceptibilities (Caspar *et al*, 2017) and clinical response to antibiotic therapy since the introduction of fluoroquinolones in the treatment of tularemia. From these data, it can be stressed that ciprofloxacin is the main effective oral therapeutic option for mild forms of tularemia, while doxycycline (a bacteriostatic antibiotic) is associated with higher treatment failure rates. We recommend that ciprofloxacin (or another effective fluoroquinolone) be used as first line drug in tularemia cases of mild to moderate severity. Doxycycline should only be used as an alternative when fluoroquinolones cannot be administrated because of intolerance or contra-indication.

P1-12
Advanced development of a novel live vaccine strain against tularemia

Wayne Conlan[1], Anders Sjöstedt[2], Qing Liu[3], Herbert Gelhaus[4]
[1]National Research Council Canada, Ottawa, Ontario, Canada, [2]Umeå university, Umeå, Sweden, [3]National Research Council Canada, Ottawa, Canada, [4]MRIGlobal, Kansas City, MO

Francisella tularensis (*Ft*) subspecies *tularensis* (*Ftt*) is extremely deadly for humans when inhaled. It was one of the first pathogens to be weaponized to a battlefield ready state. *Ft* live vaccine strain (LVS) derived from a subspecies *holarctica* isolate is the sole vaccine evaluated for efficacy in humans. Its efficacy against dermal challenge with *Ftt* strain, SCHU S4, was remarkable, but was sub-optimal against aerosol challenge. “Clinical” lots of LVS were first produced 70 years ago, but it remains unlicensed by vaccine regulatory authorities for various reasons. For humans, LVS is recommended only for administration by scarification. Moreover, over the past three decades a comprehensive description of the human immune response to LVS administered this way has accumulated. In contrast, nothing is known about human immune responses to LVS delivered by alternative routes of administration. This could severely hamper bridging studies under the FDA Animal Rule, since the immunity elicited by alternate vaccination routes in animals has no comparators in humans. We hypothesized that a live vaccine based on SCHU S4 might provide better protection than LVS due to the presence of *Ftt*-specific protective antigens. We generated ~100 targeted gene deletion mutants of SCHU S4, and screened them for decreased virulence and enhanced efficacy versus LVS against respiratory challenge with *Ftt* strains in mice. Only one mutant, SCHU S4 *ΔclpB* (*ΔclpB*) fit the bill. Deleting addition virulence genes from *ΔclpB* reduced both pathogenicity and efficacy. For the past 8 years we have been studying why *ΔclpB* outperforms LVS in animals using a variety of immunological, proteomic, and genomic techniques. These, and other parameters are being developed into robust lot release assays. Additionally, we have generated safety data sufficient to have *ΔclpB* removed from the CDC Select Agent List. Furthermore, we have developed a scale-up process to produce 2-4 million doses from an overnight 25L fermenter run, and a lyophilisation method that maintains the viability and immunogenicity for fermenter-grown *ΔclpB* when stored at +4°C or lower for at least 3 years. Details of the key steps in the ongoing development of *ΔclpB* towards IND-ready status will be presented.

P1-13
targeting the host response to treat *Francisella tularensis*

Riccardo D'Elia[1], Joshua Casulli[2], Graeme Clark[3], Diane Williamson[3], Tracy Hussell[2], Mark Travis[2]
[1]Defence Science and Technology Laboratory, Salisbury, United Kingdom, [2]University of Manchester, Manchester, United Kingdom, [3]DSTL, Salisbury, United Kingdom

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. Recently we have explored the role of specific cell populations and regulators, important in maintaining immune homeostasis, during *F. tularensis* infection. We have demonstrated, through the use of KO mice, that manipulation of the Regulatory T cell network and the CD200R pathway, that the pathogenesis of disease can be altered by changing the overall cytokine profile. Identification of key immune cells/ molecules such as these are essential in generating novel therapeutics to not only treat intracellular pathogens, but also reduce the emergence of antimicrobial resistance.

P1-14
Molecular evidence of *Francisella tularensis* in nature, 10 years after a large outbreak of tularemia in Spain

Dolors Vidal[1], Francois Mougeot[2], Ruth Rodríguez-Pastor[3], Fernando Jubete[4], Beatriz Arroyo[2], Rosa González Martín-Niño[5], Isabel Jado[5], Juan José Luque-Larena[3], **Raquel Escudero**[6]

[1]Facultad de Medicina, Área Microbiología, Universidad de Castilla La Mancha, Ciudad Real, Spain, [2]Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain, [3]Dpto. Ciencias Agroforestales, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid; Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain, [4]Dpto. Ciencias Agroforestales, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid, Palencia, Spain, [5]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain, [6]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain

Background and aim: Tularemia is a zoonosis caused by *Francisella tularensis*, a pathogen that can use a variety of transmission routes in nature as well as in humans. Since 1997 more than 1,000 human cases of tularemia have been declared in Spain in two major outbreaks, but knowledge about the epidemiology of this infection is very limited. The ability of *F. tularensis* to find mechanisms to persist in the environment and cause new outbreaks makes it necessary to identify, among others, the potential reservoirs during inter-epizootic periods, the main objective of this study. **Material and methods:** The sampling points were located in an area of Castilla y León, well known as "hot spots" for tularemia. They corresponded to surface water and sediments, as well as crayfish, mosquitoes, hares and ticks. Specifically, 8 zones divided into 4 subzones were sampled, including three rivers, three irrigation channels and three lagunes. Samples were collected in July and November of 2016 and 2017. For detection of *Francisella* *lpn*A was amplified by conventional PCR and hybridization by reverse line blotting with specific probes. Positive samples were confirmed by a real-time multitarget TaqMan PCR, using *tul*4 and *ISFtu*2 assays. For additional characterization *sdh*A gen and VNTR Ft-M19 were amplified and sequenced.

Results: A total of 102 samples of water, 111 sediments, 343 crayfish, 156 mosquitos, 64 hares and 126 ticks from 14 hares were collected. Overall, 2 samples of water, 3 of sediments, 7 hares and 17 ticks tested positive for *Francisella*. Interestingly, *F. tularensis* subspecie *holarctica* was detected in water, hares and ticks while *F. hispaniensis* was the species detected in water and sediments.

Conclusions: In this study we provide evidence of *Francisella* presence in nature during periods in which no large outbreaks of tularemia occur and suggest that hares and ticks play an important role in the maintenance of the bacteria in the terrestrial environment. Moreover, to the best of our knowledge this is the first description of *F. hispaniensis* in abiotic environment, which enforces the need to assess the risk for the population and establish the necessary measures for its prevention and control.

P1-15
Efficacy of the *Francisella tularensis* SCHU S4 Δ*clpB* Vaccine Strain in a Fischer 344 Rat Inhalational Tularemia Model

Herbert Carl Gelhaus[1], Bill Sosna[2], Daniel Boan[3], Cheryl Nevins[2], Paul Anderson[4]
[1]MRIGlobal, Kansas City, MO, [2]MRIGlobal, Kansas City, [3]Kansas City University of Medicine and Bioscience, Kansas City, [4]University of Missouri, Columbia

F. tularensis (***Ft***), the causative agent of tularemia, is an infectious bacterium of interest due to its potential use as a biological weapon. However, despite decades of research, a tularemia vaccine has not been licensed. We have contributed to the advanced development of a deletion mutant of the Type A ***Ft*** strain SCHU S4 missing the *clpB* gene (Δ*clpB*) as a live vaccine candidate. Previous research in mice demonstrated that Δ*clpB* is less reactogenic and more efficacious than the live vaccine strain (LVS). However, it is likely that FDA licensure of Δ*clpB* for the prevention of tularemia will require efficacy data in the Fischer 344 rat model. We confirmed that our ***Ft*** SCHU S4 cell bank had equivalent lethality to Dr. Terry Wu's Ft SCHU S4 cell bank when injected intraperitoneally in Fischer 344 rats. We then determined the median lethal dose of aerosolized ***Ft*** SCHU S4 to be 9.32 cfu/animal in our aerosol system. With the model established, we examined dose sparing of the Δ*clpB* vaccine and the breakthrough challenge dose of ***Ft*** SCHU S4 in rats vaccinated with Δ*clpB.* We examined Δ*clpB* duration of protection at 108 and 365 days post-vaccination. All intradermal (ID) doses of Δ*clpB* protected all rats from challenge 42 days post-vaccination with 650 cfu/rat of aerosolized ***Ft*** SCHU S4, with the lowest examined dose being 1×10^3 cfu/rat. Rats vaccinated with 1×10^5 cfu/rat challenged with ~6×10^3 cfu aerosolized ***Ft*** SCHU S4 all survived, while rats challenged with higher doses all died. An additional cohort of rats vaccinated with 1×10^5 cfu/rat Δ*clpB* were challenged 98 days post-vaccination with ~650 cfu/rat and all vaccinated rats survived. Additional cohorts are awaiting challenge to determine the duration of protection. In conclusion, the Δ*clpB* vaccine performs very well in the rat tularemia model and we plan to further develop the vaccine for FDA licensure.

P1-17
Development of a phage integration vector for *Francisella*

Hana Tlapák, Kristin Koeppen, Kerstin Rydzewski, Roland Grunow, **Klaus Heuner**
[1]*Robert Koch Institute, Berlin, Germany*

Introduction: *Francisella tularensis* (*Ft*) is the causative agent of tularemia. It can be found in a wide spectrum of wild. In a recent study, we identified and described the genomic island (GI) FhaGI-1 located within the genome of *Francisella hispaniensis* (*F. tularensis* subsp. *novicida*-like 3523). We recently showed that its *att* sites in combination with the site-specific integrase are sufficient to generate the episomal form of the GI in *E. coli* and *Ft.* subsp. *holarctica*.Using our knowledge about the genomic island FhaGI-1, we decided to generate a phage integration vector which is stable in *Francisella* without selective pressure. **Results:** In this study, we constructed two variants of a *Francisella* phage integration vector, named pFIV1-Val and pFIV2-Val, using the *attL/R*-sites and the site-specific integrase (FN3523_1033) of FhaGI-1, a chloramphenicol resistance cassette, a multiple cloning site and a *sacB* gene for counter selection of transformants against the vector backbone (pUC57-Kana). The vector pFIV1-Val generates a circular episomal form in *E. coli*. pFIV1-Val was used to transform *Ft. holarctica* LVS where FIV-Val stably integrated site specifically into the $tRNA^{Val}$ gene of the genome, and pUC57-Kana is lost due to counter selection. Using an anti-Gfp antiserum, we demonstrated that a 'gene of interest', cloned into the MCS of pFIV1-Val is expressed. The functionality of the new vector was further demonstrated by the successful complementation of intracellular replication of a *Francisella* D*iglC* mutant strain in human U937 cells. The vector was stable *in vitro* and during host-cell infection without selective pressure. Moreover, we used the vector to successfully integrate a *gfp* gene into other *Francisella* species (*Ft. novicida* Fx1 and U112, *and Francisella* sp. strain W12-1067). Then we cloned a *sacB*-gene into the non-integrating part of the vector, to allow for easier selection of clones with only the integrated construct present but not the "empty" pUC57-Kan vector, leading to the second *Francisella* integration vector pFIV2-Val.

Conclusion: Thus, the vectors pFIV1-Val and pFIV2-Val can be applied as a further genetic tool in *Francisella* research, expanding the present genetic tools by an integrative element. The element is suitable to perform long-term experiments with different *Francisella* species.

P1-18
Whitebark pine extract augments immune responses that diminish bacterial infection

MacKenzie Jacobs[1], Elliot Collins[2], James Healy[3], Roger Seeber[4], Joseph Horzempa[5]
[1]West Liberty University, New Cumberland, WV, [2]West Liberty University, West Liberty, WV, [3]West Liberty University, West Liberty, IN, [4]West Liberty University, West Liberty, [5]West Liberty University, West Liberty, WV, WV

Francisella tularensis is a highly infectious bacterium that causes tularemia. Currently, there are no legal treatments in the United States. Because of the highly infectious nature of *F. tularensis*, this organism can be used in bioterrorism. Therefore, to counteract this potential threat, development and discovery of novel treatments is highly desired. During human infection, *F. tularensis* replicates in phagocytic cells of the immune system such as monocytes and macrophages. Through a high throughput screen, we previously identified that Whitebark Pine extract (Pinus albicaulis) inhibited replication *F. tularensis* within monocytes. Bio-assay guided fractionation was used to isolate the compounds responsible for the aforementioned activity. Here, Galleria mellonella larvae were infected with *F. tularensis* LVS (an attenuated type B strain) and were treated with fractions of the Whitebark Pine extract. Infected larvae treated with one such fraction (referred to as Pa-9) exhibited significant survival compared to the mock-treated insects. Similar results were observed in chicken embryos infected with *F. tularensis* LVS that had been treated with Pa-9 suggesting that this fraction contained the active compound. To determine whether Whitebark Pine Extracts were directly antibacterial, an antibiotic disk-diffusion assay was used. None of the fractions tested inhibited the growth of bacteria outside the context of infection, indicating that this extract does not contain traditional antibiotic compounds. The further separation of Pa-9 and identification of the active compound is ongoing.

P1-19
Variable-number tandem repeats and whole-genome single-nucleotide polymorphisms in *Francisella tularensis*, Spain 1997-2014

Kerstin Myrtennäs[1], Raquel Escudero[2], Miklós Gyuranecz[3], Anders Johansson[4]
[1]Swedish Defence Research Agency (FOI), Umeå, Sweden, [2]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain, [3]Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary, [4]MIMS, Clinical Microbiology, Umeå University, Umeå, Sweden

Background. Previous studies indicated that *F. tularensis* subspecies *holarctica* causing tularemia in Spain entered this country recently with the first outbreaks reported 1996-1997. Variable-number tandem repeats (VNTRs) or whole-genome single-nucleotide polymorphisms (SNPs) are often used for molecular epidemiology studies of tularemia. **Objectives.** Because *F. tularensis* in Spain is an ideal case for investigating recent molecular evolution, we explored the relationship between the two types of genetic markers. In addition, we investigated if VNTR copy-numbers could be predicted using short-read whole-genome sequence (WGS) data.

Materials and Methods. Eight VNTR-markers were sized in the genomes of 11 strains from 2014 using capillary electrophoresis. The same markers were predicted using Illumina WGS data. The agreement between sizing by capillary electrophoresis and prediction from WGS data was analyzed. Genetic relationships among 36 strains from Spain 1997-2014 and two reference strains, from France and Italy, were inferred using SNPs and phylogenetic analyses. The phylogeny was compared with WGS-predicted VNTR-markers.

Results. We found an excellent correlation between VNTR-marker sizing by capillary electrophoresis and prediction from WGS data for 9/11 markers in the test set of 11 strains. The prediction failed for 2/11 markers in about 40% of the cases because the VNTRs were positioned adjacent to large insertions-sequence elements at the end of contigs in the WGS data. Two out of the 11 VNTR-markers (marker Ft-M3 and Ft-M4) were variable among the strains. We identified 120 informative SNPs in a 1,659,961-nucleotide alignment of the WGS data of 38 genomes. The strains from Spain formed a star-like SNP phylogeny with 6 branches emerging from a common node indicating recent clonal expansion. The strains clustered into 23 SNP-genotypes and 5 VNTR-genotypes (Ft-M3/Ft-M4-combinations). The clustering of strains by VNTR-markers, in general, agreed with the clustering by SNPs, but whole-genome SNPs provided higher strain resolution.

Conclusions. VNTR-markers can be predicted using Illumina WGS data confirming that these data are useful for molecular epidemiology studies of tularemia based on VNTR analysis. Agreement between strain clustering using whole-genome SNPs or VNTRs suggests that both genetic marker types reflect the recent evolution of *F. tularensis*. Generally, whole-genome SNPs provide higher strain resolution.

P1-20

Francisella tularensis encodes a novel lipoprotein required for efficient growth under iron limiting conditions.

Todd Kijek[1], Joel Bozue[2], Shaun Steele[3], Thomas Kawula[4]
[1]USAMRIID, Ft. Detrick, MD, [2]USAMRIID, Ft Detrick, [3]Washington State University, Pullman, [4]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA

Francisella tularensis is a highly pathogenic bacterium, whose ability to cause disease in animals is intimately tied to its ability to invade and replicate within infected host cells. Despite its relatively small genome, *Fransicella* contains several novel genes that are necessary to support this unique intracellular life cycle. One such gene is FTL_0073, which is predicted to encode a protein of unknown function within the Live Vaccine Strain (LVS) of *Francisella*. Orthologs of this gene in both the Schu4 (FTT_1676) and *F. novicida* (FTN_0109) strains of *Francisella* are known to be required for virulence in both *in vivo* and *in vitro* models of infection. While this locus is highly conserved within *Francisella* sp., bioinformatic based searches do not reveal any obvious homologs to this protein in other bacterial species. In an effort to elucidate a potential function for this gene in virulence, we examined the contribution of FTL_0073 to the pathogenesis of the LVS of *Francisella*. Similar to published results, deletion of FTL_0073 impairs the ability of LVS to replicate within both *in vitro* and *in vivo* models of infection. Using chemically defined medium, we discovered that growth of the FTL_0073 mutant was severely reduced compared to *wt* LVS under iron limiting conditions. Additionally, this mutant displayed increased sensitivity to dipyridyl and deferoxamine, two known iron chelators. Interestingly, when the available pool of extracellular iron was held predominantly in the ferrous state, the mutant was able to grow similarly to *wt* LVS at decreased concentrations of extracellular iron. Growth defects of the mutant strain could not be attributed to loss of siderophore production as both the FTL_0073 mutant and *wt* LVS produced similar amounts of siderophore as measured by chrome azurol sulfonate assay. Cross feeding experiments demonstrated that the FTL_0073 mutant was able to promote growth of *wt* LVS, however, *wt* LVS did not promote growth of the FTL_0073 mutant. Collectively, these results demonstrate that FTL_0073 is required for *Francisella* to efficiently acquire iron from the extracellular environment and suggest that its function relates to siderophore dependent iron acquisition.

P1-21
Francisella responds to *Burkholderia* diffusible signal factor (BDSF) by increasing siderophore and chitinase expression and dispersing biofilm.

Monique van Hoek, George Mason University, Centreville

In many bacteria, the ability to modulate biofilm production relies on specific quorum-sensing signaling molecules, either self-produced or made by neighboring microbes within their ecological niche. Such signaling molecules have not yet been characterized in the genus *Francisella.* We analyzed the signaling effect of *Burkholderia* Diffusible Signal Factor (BDSF) on *F. novicida,* a model organism for *F. tularensis,* and demonstrated that BDSF both inhibits the formation of and causes dispersion of *Francisella* biofilm. 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 relA and chitinases. Specifically, BDSF caused an increase in RelA expression and increased levels of (p)ppGpp, leading to decreased biofilm production. In addition, expression of the chitnases ChiA, ChiB and CBP (Chitin binding protein) were highly upregulated. Using a chitinase inhibitor, the anti-biofilm activity of BDSF was also shown to be chitinase-dependent. ChiA and ChiB mutants were shown to be deficient in biofilm production. These results support our observation that exposure of *F. novicida* to BDSF causes biofilm dispersal. Furthermore, BDSF treatment upregulated the expression of genes involved in iron acquisition (*figABCD)*, increasing siderophore production. Thus, this study provides the first evidence for a potential role and mechanism of DSF signaling in the genus *Francisella* and also suggests the possibility of interspecies signaling between *Francisella* and other bacteria.

P1-22
The use of Nanotrap microparticles to enhance detection of *Francisella* antigens.

Monique van Hoek, George Mason University, Centreville

Tularemia diagnostics and detection currently face a number of challenges, including difficulty culturing, cross-reactivity of serological tests and low sensitivity during the early stages of infection. To help address these problems we have tested a range of Nanotrap particles, hydrogel microparticles with varying chemical-affinity baits and a demonstrated ability to bind, sequester and enrich low-abundance biomarkers from complex biological samples. These particles have the potential to greatly improve the sensitivity and accuracy in detecting and diagnosing Tularemia. Prior research has shown Nanotrap particles' ability to bind and enrich biomarkers of a number of different infectious agents, including: *Borrelia burgdorferi*, Rift Valley Fever Virus and *Mycobacterium tuberculosis*. In this study we tested the ability of different Nanotrap particles for their ability to bind to *Francisella* antigens via western blotting and proteomics (LC/MS/MS) analysis. The particles ability to bind and enrich was tested in both aqueous buffers and from blood/serum samples. We characterized the full range of *Francisella* proteins bound to these particles compared to the overall composition of the bacteria using proteomics. We found that two Nanptrap particle types (CN3110 and CN3140) were able to strongly bind Tul4/LpnA, a 17 kDa highly immunogenic lipoprotein found in all *Francisella* species. The successful Nanotraps both contain Cibacron Blue FG3A as their affinity bait inside the particle core, and one has an external sieving shell that prevents larger molecular weight biomolecules from binding to the core. These particles have the ability to greatly increase the sensitivity and accuracy of Tularemia detection and diagnosis.

P1-23
Efficacy of a Live Attenuated *F. novicida* Tularemia Vaccine Expressing OAg^{FTT}

Aanuoluwa Adelani[1], Xhavit Zogaj[2], Karl Klose[3], Jesse Nguyen[4]
[1]University of Texas at San Antonio, San Antonio, TX, [2]The University of Texas at San Antonio, San Antonio, TX, [3]University of Texas San Antonio, San Antonio, TX, [4]Univeristy of Texas at San Antonio, San Antonio, TX

Fransicella tularensis subsp. *tularensis (Ftt*) is an intracellular gram-negative bacterium that causes tularemia, a fatal disease in humans and animals. *Ftt* is classified as a Category A select agent and potential bioweapon due to its low infectious dose and high mortality rate. *F. novicida (Fn),* which is a closely related species to *Ftt,* causes a lethal disease in mice but is avirulent in healthy humans. Thus, *Fn* is being developed as a live vaccine to protect against Tularemia. We have previously demonstrated that vaccination with *Fn* containing a deletion of the Type VI secretion gene *iglD* (Fn *iglD*) protects Fischer 344 rats and cynomolgus macaques against pulmonary challenge with *Ftt.* We further modified the Fn *iglD* vaccine strain to express the LPS O-Antigen from *Ftt* (Fn *iglD* OAg^{FTT}*;* KKF768), in order to enhance its ability to induce humoral immunity against Ftt. Vaccination of Fischer 344 rats intratracheally with KKF768 (prime/boost) induced elevated serum antibodies against OAgFTT. An intratracheal prime/boost vaccination regimen of rats with KKF768 provided protection (88%) against pulmonary challenge with the virulent Ftt Schu S4 strain 10 weeks post-vaccination. Protection against Ftt could be induced by both intratracheal and intramuscular delivery of KKF768, but not by oral vaccination. These results indicate that KKF768 (Fn *iglD* OAg^{FTT}*)* is a potential vaccine candidate against tularemia.

P1-24

Interplay of virulence and resistance in *F. tularensis*: Ciprofloxacin resistance acquisition coincides with spontaneous mutations in LPS biosynthesis and transport genes

Beth Bachert[1], Fabrice Biot[2], Ronald Toothman[3], Sherry Mou[4], Sabrina Rodriguez[3], Jason Ladner[3], Galina Koroleva[3], Sean Lovett[3], Gustavo Palacios[3], Patricia Worsham[5], Joel Bozue[5]

[1]United States Army Medical Research Institute of Infectious Diseases, Frederick, [2]Institut de Recherche Biomédicale des Armées, Département de Biologie des Agents Transmissibles, Brétigny-sur-Orge, France, [3]U.S. Army Medical Research Institute of Infectious Diseases, Frederick, [4]USAMRIID, Ft. Detrick, [5]USAMRIID, Ft Detrick

Francisella tularensis, the causative agent of tularemia or "rabbit fever", is a Gram negative bacterium that poses a significant risk as a potential biothreat agent, and the possible use of antibiotic-resistant variants of *F. tularensis* is a significant concern. Here we investigate the impact of resistance to the clinically-relevant antibiotic ciprofloxacin on virulence of the live vaccine strain (LVS) of *F. tularensis*, with a focus on identifying new targets for countermeasures. In order to assess how naturally-acquired antibiotic resistance affects virulence of *F. tularensis*, a series of ciprofloxacin-resistant (CipR) LVS strains was obtained by passaging the parent on increasing concentrations of antibiotic. Whole genome sequencing of CipR variants revealed mutations in genes directly linked to antibiotic resistance such as *gyrB* and *parE*. Interestingly, additional mutations were identified in genes encoding putative virulence factors, including the lipopolysaccharide (LPS) biosynthesis and transport genes *wbtC* and *lptE*. Correspondingly, highly resistant CipR mutants exhibited altered LPS and capsule profiles as assessed by western blotting. A mouse model of intranasal infection was used to assess the virulence of two CipR variants, Cip80 10-1 and Cip128 10-3, harboring the fewest and most mutations, respectively. Cip80 10-1 was slightly attenuated while Cip128 10-3 was completely attenuated in mice. Notably, the Cip128 10-3 strain harbored mutations in both the *wbtC* and *lptE* genes. To determine the effect of individual genes on virulence, *ΔlptE* and *ΔwbtC* mutants were generated in LVS via group II intron interruption. Production of LPS and capsule was diminished in the *ΔwbtC* mutant, but unaffected in the *ΔlptE* mutant. The *ΔwbtC* mutant was significantly attenuated in macrophages and completely attenuated in mice, even for doses greater than 100,000 CFU, demonstrating *wbtC* as critical for virulence in LVS. Complementation of the *ΔwbtC* mutant restored both production of LPS and capsule and virulence in mice to WT levels. In contrast, *ΔlptE* was not affected in its ability to replicate in macrophages but showed significant attenuation in mice that was restored by complementation. Overall, this work highlights LPS biosynthesis and transport genes as key virulence factors and potential targets for countermeasures against *F. tularensis*.

P1-25
Outer membrane vesicles and nanotubes in *Francisella tularensis* – proteomic characterization and role in host-pathogen interaction

Jana Klimentova[1], Ivona Pavkova[2], Lenka Plzakova[3], Jan Bavlovič[1], Klara Kubelkova[2], Jiri Stulik[4]
[1]University of Defense, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [2]University of Defence, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [3]University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove, Czech Republic, [4]Faculty of Military Health Sciences, Hradec Kralove, 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 *Francisella* spp. apart from the classical spherical OMV produces also less usually shaped outer membrane nanotubes. In this study OMV were isolated from culture medium of *F. tularensis* subsp. *holarctica* FSC200 (a clinical isolate) by medium concentration followed by high-speed centrifugation and density gradient purification. Transmission electron microscopy of the low density fraction revealed a rich mixture of vesicles (spherical and tubular all together). Proteomic analysis of the purified OMV fraction resulted in ca 300 proteins. This fraction was rich in outer membrane proteins, lipoproteins, immunoreactive proteins as well as previously described virulence factors. By comparing the proteomic composition of OMV with the membrane enriched fraction we have detected proteins specifically concentrated in OMV.

The interaction of purified OMV with murine primary bone marrow-derived macrophages (the predominant host cell type for *F. tularensis*) was visualized by fluorescence microscopy. Rapid entry of OMV into the cells was observed as well as prolonged viability of the macrophages which suggests their role in host-pathogen interaction.
We have also isolated OMV from bacteria cultivated *in vitro* under several cultivation conditions that simulated the diverse conditions of the *F. tularensis* life cycle. These included conditions mimicking the environment inside the mammalian host cell (oxidative stress, low pH, elevated temperature) and low temperature that mimicked the external milieu. Low pH and high temperature lead to the most prominent increase of OMV production. Moreover, OMV isolated in high temperature contained extremely long nanotubes in comparison with other cultivation conditions. Semi-quantitative proteomic comparison of OMV derived from different cultivation conditions resulted in distinct groups of proteins characteristic for each stress.
The study was financially supported by the Grant Agency of the Czech Republic (project No. 17-04010S) and Ministry of Defense of the Czech Republic (Long-term organization development plan Medical Aspects of Weapons of Mass Destruction).

P1-26
Prevalence of *Francisella tularensis* among small rodents in continental Croatia

Mirna Mihelcic[1], Josipa Habus[2], Marko Vucelja[3], Valentina Marecic[4], Petra Svoboda[5], Mihaela Matovina[4], Ivan Kurolt[5], Mateja Ozanic[4], Alemka Markotic[5], Nenad Turk[2], Josip Margaletic[3], Marina Šantić[1]
[1]Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia, [3]Faculty of Forestry, University of Zagreb, Croatia, Zagreb, Croatia, [4]Faculty of Medicine University of Rijeka, Rijeka, Croatia, [5]University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia

*Francisella tularensis*is a causative agent of zoonotic disease tularemia infecting wide range of hosts: arthropods, mammals and birds. In this study, the prevalence of rodent's tularemia in Croatia was investigated. The 456 samples of small rodents were collected in a 7 different localities in continental Croatia during 2-year study. Spleen samples of: 197 *Apodemus agrarius* (striped field mouse), 78 *Apodemus sylvaticus* (wood mouse), 92 *Apodemus flavicollis* (yellow-necked mouse), 17 *Myodes glareolus* (bank vole), 27 *Mycrotus agrestis* (field vole), 20 *Microtus arvalis* (common vole), 13 *Sorex araneus* (common shrew), and 12 samples of unidentified species were investigated for the presence of DNA of *Francisella* spp. using qRT-PCR method. Two mice of *Apodemus agrarius* species and one *Apodemus sylvaticus*, originated from the same area - locality of Lipovljani, were found to be positive on *Francisella*, revealing the presence of bacteria among small rodents population in Croatia.

P1-27
Bioorthogonal labeling of *F. tularensis* proteome during *in vitro* infection

Klara Kubelkova[1], Petra Spidlova[2], **Ivona Pavkova**[1], Jiri Stulik[3], Juraj Lenco[1]
[1]University of Defence, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [2]Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic, [3]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

The pathogenic strains of *Francisella tularensis* use sophisticated but still unclear mechanisms to evade host cell defenses and to exploit the intracellular space for their effective replication. The analysis of bacterial proteins expressed or secreted during host cell infection might significantly contribute to their elucidation. However, the identification of low abundant bacterial proteins from the complex pathogen-host lysates is highly challenging especially due to the large excess of eukaryotic proteins. The recently developed method, termed bioorthogonal noncanonical amino acid tagging (BONCAT) represents a very promising way to differentiate bacterial proteins from host proteomes. The introduction of mutant form of the methionyl-tRNA synthetase (NLL-MetRS) into the bacteria enables the incorporation of azide-functionalized methionine surrogate (e.g. azidonorleucine - ANL) selectively into the bacterial proteins. In the subsequent click chemistry reaction, the azide-labeled proteins are covalently coupled to an alkyne-bearing affinity tag, enriched by affinity purification and subjected to MS/MS identification. Here we present a workflow for analysis of *F. tularensis* secretome in infected macrophages based on the BONCAT technology. For this purpose we first introduced a gene encoding NLL-MetRS to the virulent *F. tularensis* subsp. *holarctica* FSC 200 strain. The resulting mutant strain was then successfully tested for its ability to introduce ANL into the newly synthesized proteins. The murine monocyte-macrophage cell line J774.1 will next be infected with the NLL-MetRS mutant strain in the presence of ANL in the cultivation medium. In the selected time post-infection, host cells will be selectively lysed keeping the bacteria intact and the bacterial cells will be removed by centrifugation and filtration. Subsequently the ANL-labeled proteins will be enriched using the click chemistry tagging reaction followed by affinity purification and mass spectrometry identification. We have so far realized number of preliminary experiments to ascertain the optional conditions for each of the proposed steps. This approach should be widely useful for the identification of determinants of *F. tularensis* pathogenicity.

This work was supported by a Ministry of Defence of the Czech Republic - Long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence.

P1-28
Protection of vaccinated mice against pneumonic tularemia is associated with an early memory sentinel-response in the lung

Liat Bar-On[1], Hila Cohen[2], Uri Elia[3], Shahar Rotem[3], Adi Kinori[2], Erez Bar-Haim[2], Theodor Chitlaru[3], Ofer Cohen[2]
[1]Israel Institute for Biological Research, Ness-Ziona, Israel, [2]IIBR, Ness-Ziona, Israel, [3]IIBR, Ness Ziona, Israel

The importance of the lung cellular immunity in vaccine-mediated protection against tularemia is a controversial matter. Live attenuated vaccine strains of *F. tularensis* such as LVS (Live Vaccine Strain), elicit an immune response protecting mice against subsequent challenge with the virulent SchuS4 strain, yet the protective immunity against pulmonary challenge is limited in its efficacy and longevity. We established a murine intra-nasal immunization model which distinguishes between animals fully protected, challenged at 4 weeks post double-vaccination (200 inhalation Lethal Dose 50%, LD_{50}, of SchuS4), and those which do not survive the lethal SchuS4 infection, challenged at 8 weeks post double vaccination. Early in the recall immune response in the lung (before day 3), disease progression and bacterial dissemination differed considerably between protected and non-protected immunized mice. Pre-challenge analysis, revealed that protected mice, exhibited significantly higher numbers of lung *Ft*-specific memory T cells compared to non-protected mice. Quantitative PCR analysis established that a higher magnitude, lung T cells response was activated in the lungs of the protected mice already at 24 hours post-challenge. The data imply that an early memory response within the lung is strongly associated with protection against the lethal SchuS4 bacteria presumably by restricting the dissemination of the bacteria to internal organs. Thus, future prophylactic strategies to countermeasure *F. tularensis* infection may require modulation of the immune response within the lung.

P1-29

Genetic analyses of *Francisella tularensis* in Fennoscandia reveal distinct subclade distribution and closely related isolates with large temporal and geographic distances.

Dawn Birdsell[1], Kerstin Myrtennäs[2], Elina Tonteri[3], Susanna Sissonen[4], Sandra Helland[5], Anders Johansson[6], Malin Granberg[7], Heidi Hemmilä[3], K Pelkonen[8], Carola Grub[9], Simo Nikkari[3], Mats Forsman[7], DAVID WAGNER[10]

[1]Northern Arizona University, Flagstaff, AZ, [2]Swedish Defence Research Agency (FOI), Umeå, Sweden, [3]Centre for Military Medicine and for Biological Threat Preparedness, Helsinki, Finland, [4]Centre for Biological Threat Preparedness, National institute for Health and Welfare, Helsinki, Finland, [5]Norwegian armed forces medical services, JESSHEIM, Norway, [6]MIMS, Clinical Microbiology, Umeå University, Umeå, Sweden, [7]Swedish Defence Research Agency, Umeå, Sweden, [8]Finnish Food Safety Authority Evira Department of Clinical Microbiology, Helsinki, Finland, [9]Norwegian Armed Forces Medical Services, Institute of Microbiology, Oslo, Norway, [10]Northern Arizona University, FLAGSTAFF, UNITED STATES

Incidences of human tularemia in Fennoscandian countries (Norway, Sweden, and Finland) are among the highest in the world. Infections in Norway appear to be mainly water-borne, whereas in Sweden and Finland, they are primarily vector-borne; the basis for these differences is largely unknown. *Francisella tularensis* isolates from Sweden have undergone extensive genetic study, revealing high levels of phylogenetic diversity and new insights into its ecological survival, but less is known of *F. tularensis* from Norway and Finland. Our objective was to determine commonalities and differences of *F. tularensis* isolated across Fennoscandian countries. We genetically classified 321 Fennoscandian DNA samples into previously published major subclades and examined geographic and temporal patterns. Fennoscandian isolates were assigned to three major subclades (B.4, B.6, and B.12). The three countries had similar phylogenetic diversity but striking differences in the distribution of these subclades. Norwegian isolates were nearly evenly distributed among three phylogenetic subclades, whereas >91% of Finnish isolates fell into one subclade (B.12). Our findings suggest that there exists a geographic distinction in the distribution of phylogenetic subclades across Fennoscandia in a west to east direction. Perhaps this reflects historical differences in tularemia evolution and dispersal across studied regions. Analysis of whole genome sequencing data of 172 Fennoscandian isolates revealed that 83.7% (144) fell within newly identified clades that formed highly clonal complexes containing a cluster of isolates that differed by 0-5 SNPs. Sixteen clonal complexes were identified across two major subclades, four within the B.6 and twelve within B.12. Among these, seven clonal complexes had isolates that had large temporal distances (>11 years), seven clonal complexes had isolates with large geographic distances (>600 km), and five clonal complexes had isolates with large distances in both space (>600 km) and time (>11 years). This pattern is consistent with *F. tularensis* having the capacity to enter a slow or non-replicating state in the environment.

P1-30
Proteomic analysis of *F. tularensis* LVS and SchuS4 secretome in blood culture

Hila Cohen[1], Liat Bar-On[2], Uri Elia[3], Shahar Rotem[3], Theodor Chitlaru[3], Ofer Cohen[1]
[1]IIBR, Ness-Ziona, Israel, [2]Israel Institute for Biological Research, Ness-Ziona, Israel, [3]IIBR, Ness Ziona, Israel

We have documented in the past proteomic-serological identification of immuno-dominant bacterial antigens expressed upon exposure of experimental animals to *Francisella tularensis* (*Ft*), relevant for the development novel diagnostic, disease progression biomarkers and therapeutic strategies. We have now extended these studies for the detection of microbial antigens in clinical samples, as a first step in the development of novel analytical tools for rapid diagnosis of infection. Blood cultures represent one of the most common specimens for detection of *Francisella tularensis* infection, routinely performed in clinical set-ups. We have developed a series of efficient polyclonal antibodies against *F. tularensis* recognizing a broad spectrum of bacterial proteins. Using these reagents, which were demonstrated to exhibit high sensitivity and specificity in ELISA tests, we were able to immunoprecipitate secreted proteins collected from blood culture inoculated with LVS (*Ft holoarctica*) or SchuS4 (*Ft tularensis*) strains. Eighty-four bacterial secreted proteins were accurately identified by MS\MS analysis of filtered blood-cultures, 64 of which detected as early as 24 hours post-inoculation of the culture with Ft-spiked human blood. Surprisingly, only 3 proteins were identified in blood culture inoculated with SchuS4 24 hours post-inoculation. A total of 27 proteins were detected in the SchuS4 culture 72 hours post-inoculation. The vast majority of these proteins were also detected in the LVS-inoculated cultures and their secretion exhibited similar kinetics. The Ft blood-culture secretome identified and presented in this study was compared to the *in-vivo* secretome of the bacteria and may serve for the development of diagnostic tools.

P1-31
Inhibition of *Francisella tularensis* phagocytosis using a novel anti-LPS scFv antibody fragment

Uri Elia[1], Adva mechaly[2], Ron Alcalay[2], Hila Cohen[2], Eyal Epstein[2], Ohad Mazor[2], Ofer Cohen[2]
[1]IIBR, Ness Ziona, Israel, [2]IIBR, Ness-Ziona, Israel

Francisella tularensis (*Ft*), the causative agent of lethal tularemia, is a virulent Gram-negative, facultative intracellular bacterium. It has been previously demonstrated that binding of antibody-coated bacteria to the Fc receptor (FcγR), located on phagocytic cells such as macrophages and neutrophils, is a key process needed for efficient protection against *F. tularensis*. As the recognition of *Ft* at the host cell membrane is a key step in the infection process, we addressed the question whether an anti-*Ft* LPS antibody that lacks the ability to bind to the FcγR may inhibit the entry of *Ft* into the host cell. Generation of the antibodies suitable for experimentally addressing this issue included the following steps: (a) rabbit immunization methodology which has been shown to yield a robust and effective antibody-mediated immune response, (b) isolation of a specific anti-LPS high-affinity antibody, termed TL1, carried out using phage-display libraries, (c) generation of a soluble single-chain fragment (scFv) of TL1 (TL1-scFv) that comprises the VH-VL regions of the antibody and lacks the Fc region, while retaining its affinity towards *Ft.* Bacterial uptake was assessed upon infection of macrophages with LVS in the presence of either TL1 or TL1-scFv. While incubation of LVS in the presence of TL1 greatly enhanced bacterial uptake, LVS uptake was significantly inhibited in the presence of TL1-scFv. These results prompt further studies probing the therapeutic efficacy of TL1-scFv *in-vivo*, alone or in combination with antibiotic treatment.

P1-32
Virulence regulation by rfrA and rprA: two non-coding small RNAs in *Francisella*

Ky Hoang[1], John Gunn[2], Nrusingh Mohapatra[3], Harrison Harrison[4], Robert Munson[5]
[1]The Ohio State University, Columbus, oh, [2]The Ohio State University, Columbus, [3]Techulon Inc., Blacksburg, VA, [4]The Research Institute at Nationwide Children's, Columbus, [5]The Research Institute at Nationwide Children's Hospital, Columbus

---

Francisella tularensis is the facultative intracellular bacterium responsible for zoonotic disease tularemia. Comparative genomic analysis revealed that *Francisella tularensis* encoded very few transcriptional regulators, and only one classically arranged two-component regulatory system. Thus, *F. tularensis* must use other regulatory mechanisms to adapt to the environmental changes during its lifecycle in the environment and in the mammalian host. Post-transcriptional regulatory mechanisms are widespread in bacteria and RNA regulators are important factors that control gene expression in response to changing environments. In this study, *in silico* analysis revealed that the human virulent strain *Francisella tularensis* Schu S4 encodes at least 24 small RNAs (sRNAs) including *rprA* (RNA PmrA regulator) and *rfrA* (RNA FevR regulator) that are predicted to bind to the known virulent regulators *pmrA* and *fevR*, respectively. RNAseq has been performed which confirmed the presence of these sRNAs. We experimentally demonstrated that these small RNAs regulate their predicted targets and were both modulated by RNA chaperone protein Hfq. Finally, loss of *rprA* and *rfrA* attenuate survival in macrophages and virulence of *Francisella tularensis* Schu S4 in a mouse model of infection. Therefore, sRNAs of *Francisella* broaden the regulatory repertoire of this bacterium and affect its virulence functions.

P1-33
Tularemia in Germany—A Re-emerging Zoonosis

Daniela Jacob, Roland Grunow, Klaus Heuner, Mirko Faber
[1]*Robert Koch Institute, Berlin, Germany*

Here we describe tularemia in Germany which gives an example for many other European countries with a similar epidemiological situation. Germany represents a low incidence region with regard to tularemia in humans caused by *Francisella tularensis*. From 1960 to 2004, between 9 and 34 cases per 5-year intervals were reported, indicating a very low incidence or poor reporting activity. According to the infection protection act of 2001, tularemia is a notifiable disease in Germany. Between 2002 and 2016, 257 cases of tularemia were notified, corresponding to a mean yearly incidence of 0.03 cases per 100,000 population. While the disease is rare, surveillance of human cases indicates a re-emergence with an approximately ten-fold increase of notified cases in Germany in a period of 15 years (5 cases in 2002, 56 cases in 2017). Possible reasons include an increased presence of the pathogen in the environment and more frequent contact between humans and wildlife through leisure activities. However, an increased awareness of the disease and more frequent testing might have contributed to the continuous rise of notified cases. On the other hand, in patients presenting with lymphadenitis and fever, tularemia is rarely considered as a differential diagnosis by clinicians and diagnostic laboratories. Therefore it can be assumed that tularemia is subject to significant underdiagnosis and underreporting. Among the notified cases, the most frequent clinical presentations were glandular and ulceroglandular tularemia (32%). The laboratory diagnosis is often based on the detection of specific serum antibodies and/or of the *F. tularensis* DNA in clinical samples. Outbreaks or clusters of tularemia occur rarely. Subspecies *holarctica* is the only subspecies known to cause the disease in patients and animals in Germany. Only one other *Francisella* species, *Francisella* sp. isolate W12-1067 isolated from a water reservoir of a cooling tower of a hospital, has been found in Germany so far. We highlight some peculiarities of the pathogen as observed in Germany and describe the epidemiology, outbreaks and possible sources of infection, different clinical courses and aspects of diagnosis as well as underline the one-health aspect of tularemia considering this disease relevant for human, animal, and environmental health.

P1-34
Sequence comparison of *Francisella tularensis* LVS, LVS-G, and LVS-R

Sherry Kurtz[1], Alin Voskanian-Kordi[2], Roberto De Pascalis[1], Vahan Simonyan[2], Karen Elkins[1]
[1]CBER/FDA, Silver Spring, [2]HIVE/FDA, Silver Spring

Francisella tularensis is a gram-negative organism found in many regions of the world. *F. tularensis* can cause a fatal, febrile illness, though these tularemia infections are rare in the United States. However, the development of *F. tularensis* as a potential weapon of bioterrorism during the Cold War spurred the development of a live attenuated vaccine, denoted LVS, from *F. tularensis* subsp. *holarctica* in the 1960s. Two colony morphology variants, LVS-G and LVS-R, were generated from parental LVS by plate passage and by acridine orange mutagenesis, respectively. In vaccinated mice and rats, LVS-G and LVS-R exhibit altered immunogenicity and protective capacities. While the exact nature of the genetic mutations in these strains was unknown, previous studies indicated that both had altered lipopolysaccharide structures. To better understand the impact of these mutations on the immunogenicity of LVS, we sequenced the genomes of LVS-G and LVS-R as well as our parental laboratory stock of LVS, originally obtained from ATCC, and compared these to the *F. tularensis* subsp. *holarctica* LVS genome currently deposited in GenBank. Of note, investigation established definitively that the original LVS GenBank sequence was derived using bacteria from Lot 11 of the NDBR 101 vaccine stocks held by the Department of Defense and used in human vaccine trials. The results indicate that the genomic sequence of ATCC LVS is nearly identical to that of the human LVS vaccine, and a limited number of genomic mutations likely account for the phenotypes of LVS-G and LVS-R.

P1-35
The role of the chemokine receptor CCR2 in immunity to *F. tularensis* LVS is independent of any of its known ligands

Sherry Kurtz[1], Karen Elkins[1]
[1]*CBER/FDA, Silver Spring*

The trafficking of immune cells to the sites of infection is an important step toward responding to pathogen infection. CC Chemokine Receptor 2 (CCR2) is expressed by hematopoietic cells such as macrophages, and non-hematopoietic cells such as endothelial cells, and mediates cell recruitment to sites of inflammation. CCR2 is a receptor for the major ligands CCL2/7/12 (MCP-1/3/5). During infection with *F. tularensis* LVS or other intracellular bacterial pathogens, a $CD11c^{+}CD11b^{hi}Gr\text{-}1^{+}$ dendritic cell (DC) subpopulation traffics into colonized tissues. Here, we confirmed that CCR2 is essential for the recruitment of this DC population, because CCR2 knockout (KO) mice infected with LVS do not exhibit recruitment of these cells to the lung; instead, they accumulate in the bone marrow. However, other cells types are unaffected by the absence of CCR2, as the numbers of other populations of T cells, B cells, and myeloid cells were similar between infected wild type (WT) and CCR2 KO mice. Nonetheless, CCR2 KO mice exhibited an intradermal LVS LD_{50} more than 10,000 lower than that in WT mice. However, CCR2 KO mice that survived low doses of primary LVS infection subsequently survived high doses of secondary, lethal intraperitoneal LVS challenge. Further, primed LVS-immune cells from CCR2 KO mice controlled intramacrophage LVS replication in an *in vitro* co-culture assay. This suggests that the role of CCR2 is central to initiating innate host responses, but not adaptive responses. Given the importance of CCR2 in controlling LVS infection, we examined the role of each of the 3 major ligands in host responses. KO mice lacking any of the three major individual ligands (CCL2, CCL7, or CCL12) did not exhibit significant differences in the intradermal LVS LD_{50} compared to WT mice. "Triple" ligand knockout mice were generated by using antibodies to deplete CCL2 and CCL7 in CCL12 KO mice, but were not significantly different from WT mice in susceptibility to LVS infection. Therefore, the role of CCR2 in immunity to LVS is independent of the role serving as a direct receptor for any of these 3 ligands.

P1-36
Understanding the Mechanisms of Oxidative Stress Responses and Virulence of *Francisella tularensis* LVS

Zhuo Ma[1], Kayla King[1], Meenakshi Malik[2], Madeline Worden[1], Chandra Bakshi[3]
[1]Albany College of Pharmacy and Health Sciences, Albany, [2]Albany College of Pharmacy and Health Sciences, Albany, New York, [3]New York Medical College, Valhalla, NY

Francisella tularensis (*Ft*) is an important Gram-negative facultative intracellular human pathogen responsible for causing tularemia. It is also classified as a category A agent by the CDC based on its potential use as a bioterror agent. The molecular basis for the high infectivity and virulence of *Ft* is not well understood. However, the pathogenicity of *Ft* is mainly dependent on its ability to persist and replicate in phagocytic cells. Multiple antioxidant enzymes that are important defense factors against oxidative stress have been identified and implicated in pathogenesis of tularemia. Our previous study has demonstrated that some of these antioxidant enzymes are regulated by a LysR family transcriptional regulator, OxyR. In the present study, we further investigate the roles of pp(p)Gpp; the stringent response molecules produced by RelA and SpoT proteins, in the oxidative stress response and virulence of *Ft*. We generated a *relA* gene deletion mutant (Δ*relA*), a *relA*/*spoT* double gene mutant (Δ*relA*/*spoT*) and corresponding transcomplements of *Ft* LVS. These mutants were characterized for their sensitivity towards oxidants, ability to survive in macrophages and for their virulence in mice. As compared with wild type *Ft* LVS, the *ΔrelA*/*spoT* mutant grew slowly at high temperatures. The *ΔrelA*/*spoT* double mutant, which is unable to produce pp(p)Gpp, showed strong sensitivity towards oxidants; defective replication and survival in macrophages, and attenuated virulence in mice. Further studies employing RNA-seq and qRT-PCR revealed the global effect of pp(p)Gpp on the expression of antioxidant enzyme genes, multiple heat shock protein genes; and genes located on *Francisella* Pathogenecity Island. These results demonstrate that RelA/SpoT of *Ft* play an important role in providing resistance against oxidative stress and thereby facilitate intramacrophage survival and virulence in mice.

P1-37
An uncommon outbreak of tularemia after a wine grape harvest in fall 2016, Germany, and molecular identification of the outbreak 'strain' as well as of the source of infection

Roland Grunow[1], Daniela Jacob[1], Kristin Koeppen[1], Aleksander Radonić[1], Berit Haldemann[2], Philipp Zanger[3], Timo Wolf[4], Klaus Heuner[1]
[1]Robert Koch Institute, Berlin, Germany, [2]Robrt Koch Institute, Berlin, Germany, [3]Institute for Infectious Disease Prevention Landau, Federal State Agency for Consumer & Health Protection Rhineland-Palatinate, Landau, Germany, [4]University Hospital Frankfurt, Frankfurt, Germany

End of 2016, an uncommon outbreak of tularemia occurred in humans in Germanyt after a grape harvest. Six (2 females, 4 males; median age 24.5 years, interquartile range 10.3-39.5) out of 29 grape harvesters showed first symptoms (swollen cervical lymph nodes, fever, chills, difficulties swallowing, diarrhea) four to eight days after the suspected exposure and subsequently developed antibodies against the lipopolysaccharide of *F. tularensis* (detected by ELISA and Western-Blot). A multivariable analysis revealed that drinking fresh must remained the only significant predictor for acquiring tularemia (aIRR=13.5 p=0.014). Because tularemia was initially not considered, a delay of about five weeks occurred in confirming the diagnosis of tularemia. *F. tularensis* DNA could be detected using a specific real-time PCR from pus of the lymph node of 46 year old patient with a protracted course of infection. By quantitative PCR, genome equivalents were determined: In winery W1 (place of outbreak), wine sort-A (16,849 GE/ml) and sort-B (440 genome equivalents/ml) was pressed after suggesting cross-contamination on the press. Wine sort-C (1 GE/ml) from winery W2 was collected by the same mechanized harvester later that day suggesting cross-contamination via the harvester. Quantitative-PCR results revealed a substantial contamination of sort-A by 10^9–10^{10} *F. tularensis* in 730 liters. The pathogen was not detected in an unrelated sample sort-D.

A bacterial isolate could not be obtained. Using extracted DNA, *Francisella tularensis* subsp. *holarctica* belonging to the phylogenetic clade B.12/B.34 (Erythromycin-resistant Biovar II) could be identified. By direct next generation sequencing, nearly the whole genome sequence of the causative *Francisella* strain could be identified. The patient had been most likely infected with the same genotype of *Francisella* present in the wine product. Furthermore, a vertebrate-specific cytochrome b PCR identified mitochondrial DNA of *Apodemus sylvaticus* (wood mouse) in the wine products, suggesting that a wood mouse infected with *F. tularensis* may have been responsible for contaminating the must.
In conclusion, the discovered source of infection and the transmission scenario of *F. tularensis* in this outbreak have been observed for the first time and suggest the need for additional hygienic precaution measures when consuming freshly pressed must.

P1-38
Plan B: Identifying a Backup Ribosome Rescue System in *Francisella tularensis*

Tyler Goralski[1], Girish Kirimanjeswara[2], Kenneth Keiler[3]
[1]The Pennsylvania State University, State College, [2]The Pennsylvania State University, University Park, PA, [3]The Pennsylvania State University, University Park

If a bacterial ribosome reaches the 3' end of the mRNA with no stop codon, the ribosome becomes stalled in a non-stop translation complex. This is detrimental to the bacteria, which can no longer use the stalled ribosome to aid in the translation of more protein. The ability to rescue ribosomes stalled at the ends of mRNAs is essential for efficient translation, and bacteria lacking ribosome rescue die. The primary pathway for ribosome rescue is *trans*-translation, the genes for which are conserved in > 99% of sequenced bacteria. Backup systems, such as ArfA or ArfB have been identified in some bacteria, which can rescue ribosomes in the absence of sufficient *trans*-translation activity. We have previously shown that small molecule inhibitors that target all known ribosome rescue pathways can inhibit growth of *Francisella tularensis*. Interestingly, it has been shown that *trans*-translation is not critical for survival of *F. tularensis*, suggesting that this bacterium has an additional backup system, although there are no homologues to ArfA or ArfB. To identify potential backup ribosome rescue pathways, we used transposon mutagenesis followed by deep sequencing (Tn-Seq). We have identified and characterized a novel backup ribosome rescue system, alternate ribosome rescue factor T (ArfT), in *F. tularensis*, which has an amino acid sequence different from ArfA and ArfB. ArfT was found to be essential for survival in bacteria lacking *trans*-translation. Furthermore, ArfT has a distinct mechanism of action, interacting with release factor 1 (RF1), which binds to the stalled ribosome and hydrolyzes the nascent peptidyl-tRNA, whereas ArfA recruits peptide chain release factor 2 (RF2). Overexpression of ArfT rescued the growth defect observed in *F. tularensis* Live Vaccine Strain (LVS) lacking *trans*-translation, suggesting that ArfT can rescue ribosomes in the absence of *trans*-translation. Additionally, small molecule inhibitors of ribosome rescue arrest growth of *F. tularensis* LVS lacking *trans*-translation at equivalent concentrations as wild type LVS, indicating that these inhibitors also target ArfT. The identification and characterization of an alternate rescue factor in *F. tularensis* has allowed fuller insight into the physiology of ribosome rescue and has elucidated another target for ribosome rescue inhibitors.

P1-39
Epidemiological Features of Tularemia in Siberia, at the Far East, and Ural in 2005-2017

Elena Kulikalova, Sergey Balakhonov, Maria Perevalova, Andrey Mazepa, Ayuna Syngeeva
[1]*Irkutsk Antiplague Research Institute of Siberia and Far East, Irkutsk, Russian Federation*

Background. Tularemia is a natural focal zoonotic infection caused by *Francisella tularensis*. There are natural foci of tularemia in Siberia, at the Far East and in Ural; many foci are located close by large cities. **Objective.** Detection of the epidemiological characteristics of tularemia in Siberia, at the Far East and in some Ural areas (Khanty-Mansi, Yamal-Nenets Autonomous Districts and Tyumen Region) in 2005-2017.

Material/methods. Tularemia sickness rate was analyzed on the basis of the State statistical reporting «Information about infectious and parasitic diseases» and the State reports on sanitary-epidemiological well-being in the Russian Federation. The data were statistically processed using Microsoft Excel program.

Results. In Siberia there are the following natural tularemia foci: steppe, meadow-field, foothill-stream, flood land-marsh, forest, tundra types. Tundra foci are found in tundra and forest-tundra territories of Ural (Yamal-Nenets and Taimyr Autonomous Districts). There are flood land-marsh, forest and meadow-field types of natural tularemia foci at the Far East (Amur Region, Khabarovsk Territory and Jewish Autonomous Region).

Analysis of long-term dynamics of human tularemia sickness rate showed the tendency to increase of the morbidity level, with a peak in 2013 (3.25 per 100 000 population that exceeds the mean value in the Russian Federation in 4,4 times) due to high number of patients in Khanty-Mansi Autonomous District (1005 cases) through transmission from blood-sucking insects. The territorial distribution of tularemia incidence in 2005-2017 demonstrated that the highest number of the cases was found at Ural - in Khanty-Mansi Autonomous District (1048); in Siberia - in Omsk (71), Novosibirsk (53), Tomsk (2) regions and Altai (26), Tuva (2); at the Far East - in Khabarovsk (11), Primorsk (2) Territories and Sakhalin Region (9).

Conclusion. Thus, all known types of natural tularemia foci are found in Siberia, at the Far East and Ural. The most epidemically active are flood land-marsh foci. The most risk territories in Siberia are located in Novosibirsk and Omsk regions and Altai Republic; at the Far East – Khabarovsk, Primorsk Territories and Sakhalin region; at Ural – Khanty-Mansi Autonomous District.

P1-40
Five-year Study of Tularemia in Georgia

Gvantsa Chanturia[1], Roena Sukhiashvili[2], Mariam Zakalashvili[1], Ekaterine Khmaladze[1], Tea Tevdoradze[1], Ekaterine Zhghenti[1], Natalia Abazashvili[1], Irma Burjanadze[1], Nato Kotaria[1], Meri Pantsulaia[1], Gvantsa Brachveli[1], Nikoloz Tsertsvadze[1], Merab Shavishvili[1], Paata Imnadze[3]
[1]National Center for Disease Control and Public Health (NCDC), Richard G. Lugar Center for Public Health Research, Tbilisi, Georgia, [2]national center for Disease Control and Public Health (NCDC), Tbilisi, Georgia, [3]National Center For Disease Control and Public Health, Tbilisi, Georgia

The select agent Francisella tularensis is endemic in Georgia and has established ecology niche in eastern part of the country. The National Center for Disease Control and Public Health of Georgia (NCDC) carries out the regular surveillance of this especially dangerous pathogen and maintains collection of 106 strains. Intensive work was performed to study tularemia during last five year – two DTRA funded projects along with NCDC routine surveillance allowed to perform surveillance among population leaving in foci area as well as in the environment; to improve tularemia case detection by initiating active syndromic surveillance; mapping of affected with pathogen areas and making prediction models; identification of the new foci in western part of the country; genotyping of all strains and positive cases with SNP & MLVA methods; to perform whole genome sequencing and phylogenetic analysis of 20 strains from NCDC collection.

Results showed that tularemia has wider distribution throughout the country than it was known before. The main vectors of the pathogen remain the same. Genotyping of recent positive cases showed emerging of newly described among Georgian strains SNP genotype (B.Br.10). Whole genome-based SNP analysis proved these results and allowed the discovery of new diversity patterns inside the previously-established canonical SNP lineage (B.Br.027-32).

The surveillance of this pathogen is continued with improved tools for collection, detection and analysis of the results. The capacity and preparedness level for response to possible tularemia cases have significantly increased based on the work conducted in these studies.

P1-41
Rhizoferrin biosynthetic pathway in *Francisella tularensis*

Girija Ramakrishnan[1], Natalie Pérez[2], Todd Fox[2]
[1]*University of Virginia, Charlottesville, VA,* [2]*University of Virginia, Charlottesville*

*Francisella tularens*is employs a TonB-independent siderophore uptake process as one mechanism to scavenge iron from the host. Biosynthetic and transport functions for the *Francisella* siderophore, rhizoferrin are encoded by the *fslAB-CDE* operon. We, and others, have previously shown that mutants in *fslA* encoding a NIS siderophore synthetase, and in *fslC* encoding a pyridoxal-phosphate dependent decarboxylase are deficient in siderophore production. Rhizoferrin, a siderophore produced by a variety of fungi and bacteria, comprises two citrate molecules linked by amide bonds to a central putrescine moiety. To discern the steps in the *Francisella* rhizoferrin biosynthetic pathway, we tested bacterial cultures of the *F. tularensis* strain LVS and its Δ*fslA* and Δ*fslC* mutants for the ability to incorporate potential precursors from the medium into siderophore. Addition of 0.2 mM ornithine, but not putrescine, enhanced siderophore production by LVS as assayed by CAS activity in the culture supernatant. Radioactivity from U-^{14}C-ornithine was efficiently incorporated into siderophore by LVS, while ^{14}C label from 1-^{14}C-ornithine was not. Neither the Δ*fslA* nor the Δ*fslC* mutant produced any siderophore, however a potential siderophore intermediate that could be labeled by both U-^{14}C-ornithine and 1-^{14}C-ornithine was secreted into the medium by the Δ*fslC* mutant. The structure of the secreted compounds was determined by liquid chromatography using a hydrophilic C18 column coupled to a qTOF mass spectrometric analyzer. Rhizoferrin was identified in LVS culture supernatants, while citryl-ornithine was identified in the culture supernatant of the Δ*fslC* mutant. Our findings indicate that rhizoferrin biosynthesis in *Francisella* starts with FslA-mediated formation of citryl ornithine and that decarboxylation of this siderophore intermediate by FslC is necessary for a second condensation reaction with citrate to produce rhizoferrin. *Francisella* rhizoferrin biosynthesis thus appears to differ from the bacterium *Legionella pneumophila* and the fungus *Rhizopus delemar* where the proposed steps involve consecutive additions of citrate to putrescine as primary substrate.

P1-42
Engineering a targeted vaccine platform in *Francisella tularensis*

Kristen Tummillo[1], Karsten Hazlett[2]
[1]*Albany Medical College, Rensselaer,* [2]*Albany Medical College, Albany*

Francisella tularensis (*Ft*) is a gram-negative bacterium and the causative agent of tularemia. Currently, there is no FDA-approved vaccine against this potential bioweapon, however targeting of *Ft* to receptors on immune cells has shown great promise as a vaccination strategy. The goal of our work is to create an adjuvant-free *Ft* vaccine by targeting whole *Ft* to complement receptors 2 (CR2, on FDCs and B cells) and 3 (CR3, on APCs). We hypothesize that we can achieve targeting of *Ft* to CRs by genetically linking C3d to the N-terminus of an autotransporter platform and expressing this fusion protein in *Ft*. Native autotransporters are monomeric or trimeric outer membrane proteins that possess C-terminal beta barrels and surface-exposed passenger domains (N-termini); the pathway for their localization is widely conserved, suggesting that our approach could have broad applicability among gram-negative bacteria. We have modified the passenger domains of the trimeric autotransporter YadA (Yersinia enterolitica adhesion A), as well monomeric autotransporter ShdA (Salmonella adhesion) to include a FLAG-tag and a C3d derivative. When these fusions are heterologously expressed in *Escherichia coli* and *Ft* LVS, we find that our targeting proteins localize to the outer membrane with surface-accessible passenger domains. Live *Ft* LVS expressing YadA or the YadA-FLAG-C3d (YFC) fusion were tested as intranasal vaccines against pulmonary *Ft* LVS challenge in female BALB/c mice. Mice vaccinated with *Ft*-YFC displayed *Ft*-specific IgG and IgM antibody responses and significantly increased survival (100%, $p = 0.04$) over mice vaccinated with control *Ft* (80%). These results indicate that YFC expression by the vaccine strain enhances protective immune responses. Interestingly, vaccination with *Ft* expressing full-length YadA (which contains a collagen-binding domain absent in YFC) induced lower *Ft*-specific IgG responses and no significant survival advantage over the control. Going forward, we will further assess surface-exposure of the C3d moiety and binding to CRs, as well as efficacy of the vaccine strains against a more stringent *Ft* challenge. Additionally, we also seek to test inactivated forms of these targeted *Ft* strains and the applicability of this approach to other gram-negative bacteria.

Session 3

Pathogenesis and Cell Biology

S3-1
F. tularensis-infected neutrophils are Trojan Horses for macrophage infection

Lee-Ann Allen[1], Jenna McCracken[2], Ann Janowski[2], Justin Schwartz[2]
[1]*University of Iowa, Iowa City, IA,* [2]*University of Iowa, Iowa City*

We have shown that *F. tularensis* Schu S4 and LVS inhibit human neutrophil activation, escape the phagosome to replicate in the cytosol, and markedly prolong cell lifespan. Although the mechanisms of neutrophil apoptosis inhibition are partially understood, the fate of these cells is unknown. Typically, phosphatidylserine (PS) on the surface of apoptotic neutrophils triggers rapid engulfment (efferocytosis) of the dying cells by macrophages and is critical for macrophage reprogramming to a pro-resolution phenotype. As PS on *Ft*-infected neutrophils is low, we predicted that their interactions with macrophages would be impaired. In marked contrast, we discovered that binding and uptake of infected neutrophils by primary human monocyte-derived macrophages (MDMs) was significantly enhanced. Subsequent studies identified roles for CD18 and CD36, but not PS receptors, in this process. Remarkably, neutrophil uptake supported Trojan horse infection, leading to massive *Ft* replication in MDM cytosol after efferosome escape. Next, we used multiple markers to assess MDM activation state. MDMs were unpolarized at rest, and except for downregulation of the mannose receptor were largely unchanged by exposure to live *Ft* or infected neutrophils. Pretreatment of MDMs with IFNg and *E. coli* LPS induced classical M1 polarization, defined by upregulation of surface CD80, CD86 and MHC Class II, that was ablated by subsequent exposure to *Ft*-infected neutrophils. Conversely, aged, uninfected neutrophils or live *Ft* alone caused only moderate reduction of M1 markers that was not statistically significant. These data are significant for several reasons. First, the unpolarized human macrophage phenotype we report is distinct from the M2 polarization reported to occur in LVS-infected murine RAW264.7 macrophages. Second, these data are to our knowledge the first evidence that neutrophils can act as Trojan horses in tularemia. Third, our data demonstrate profound neutrophil-driven macrophage reprogramming via a mechanism that can be uncoupled from neutrophil apoptotic death. As such, our data elucidate new mechanisms for exacerbation of *Ft* infection and manipulation of the inflammatory response.

S3-2
A *Francisella tularensis* LD-carboxypeptidase is required for virulence

Briana Zellner, Jason Huntley
[1]*University of Toledo, Toledo, OH*

Studies to identify and characterize *Francisella tularensis* (*Ft*) envelope proteins are important to help understand the molecular mechanisms by which *Ft*, and other intracellular pathogens, cause disease. In addition, identification of new virulence factors may lead to the development of new therapeutics and vaccines for tularemia. We previously demonstrated that *Ft* encodes a disulfide bond formation protein ortholog, DsbA, that is required for virulence in macrophages and mice. We further demonstrated that *Ft* DsbA is a bifunctional protein that oxidizes and isomerizes complex disulfide connectivity in substrates. Using a molecular trapping approach, we identified over 50 DsbA substrates, including outer membrane proteins, known virulence factors, and many hypothetical proteins. In the current study, we selected one of these unstudied DsbA substrates, FTL1678, for detailed analysis. First, bioinformatic analyses revealed that FTL1678 contains a putative LD-carboxypeptidase domain, indicating a potential role in peptidoglycan recycling and remodeling. Second, an isogenic mutant of FTL1678 was found to be completely attenuated in a mouse pulmonary infection model, with limited lung colonization and inability to disseminate to livers or spleens. A closer examination of infected tissues indicated severe pathology in wild-type LVS-infected mice on day 5 post-infection, whereas ΔFTL1678-infected mice showed little to no pathology, similar to uninfected mice. ΔFTL1678 mutant attenuation was confirmed through *trans*-complementation, which fully-restored virulence to wild-type levels. Third, immunization with ΔFTL1678 provided significant protection against SchuS4 pulmonary challenge. Fourth, to confirm predicted roles in peptidoglycan recycling and remodeling, membrane integrity testing revealed increased resistance of ΔFTL1678 to several antibiotics and detergents. In addition, electron microscopy analysis of ΔFTL1678 demonstrated increased cell wall thickness, supporting the role of FTL1678 in cell wall remodeling. Current studies are examining ΔFTL1678 defects in macrophage intracellular trafficking/replication, assessing the function of recombinant FTL1678, testing the *in vivo* attenuation of FTL1678 active site mutants, and examining differences in peptidoglycan composition between the wild-type and ΔFTL1678. Taken together, these studies have revealed a new *Ft* virulence factor and have highlighted the importance of the *Ft* envelope in protecting the bacterium during infection.

S3-3
The *Francisella tularensis* γ-glutamyl cyclotransferase is required for virulence

Guoping Ren[1], Briana Zellner[2], **Jason Huntley**[2]
[1]New England Biolabs, Ipswich, MA, [2]University of Toledo, Toledo, OH

Studies to identify and characterize *F. tularensis* virulence factors are critically important for the development of new therapeutics and vaccines. Moreover, this information could be used to better understand how other intracellular pathogens infect host cells and evade immune detection. We have focused our efforts on studying *F. tularensis* envelope proteins because of their known roles in virulence in many other bacterial pathogens. We previously demonstrated that *F. tularensis* encodes a disulfide bond formation protein ortholog, DsbA, that is outer membrane-bound and is required for the infection of macrophages and mice. We also demonstrated that *F. tularensis* DsbA is a multi-functional protein that oxidizes, reduces, and isomerizes complex disulfide connectivity in substrates. To identify proteins that more-directly interact with host cells or that protect *F. tularensis* from immune cell damage, we used a molecular trapping approach and identified over 50 *F. tularensis* DsbA substrates, including outer membrane proteins, known virulence factors, and many hypothetical proteins. One of these hypothetical proteins, FTL1548, contains a conserved domain similar to the human gamma-glutamyl cyclotransferase (GGCT), but the role of GGCT orthologs in pathogenic bacteria remains largely unstudied. Both LVS and SchuS4 GGCT mutants were found to be fully-attenuated *in vitro* and *in vivo.* When comparing wild-type and mutant bacterial numbers in various organs after pulmonary infection of mice, 3- to 5-log reductions in were observed. Importantly, immunization with *F. tularensis* GGCT mutants protect mice against high-dose SchuS4 challenge. To confirm that FTL1548/FTT0509c has GGCT activity, we purified recombinant FTT0509c and demonstrated GGCT activity *in vitro*. Taken together, these studies have revealed a new *F. tularensis* virulence factor with GGCT activity. Studies are ongoing to understand how *F. tularensis* GGCT may play a role in glutathione degradation or to protect the bacterium from oxidative stress inside host cells.

S3-4

Inactivation of *Francisella tularensis* gene encoding putative flippase has a pleiotropic effect upon production of various glycoconjugates: evidence for PilA protein modification by O-antigen

Jiri Stulik[1], Lucie Balonova[2], Marek Link[3], Vera Dankova[3], Valeria Sesko (Sheshko)[4]
[1]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, [2]University of Defence, Hradec Kralove, Czech Republic, [3]FMHS UO, Hradec Kralove, Czech Republic, [4]Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Francisella tularensis utilizes surface glycoconjugates such as lipopolysaccharide, capsule, and capsule-like complex for its protection against inhospitable conditions of the environment. *Francisella* species also possesses a functional glycosylation apparatus by which specific proteins are O-glycosidically modified. Through the present study, we seek to extend the knowledge of protein glycosylation machinery in *F. tularensis* and examine the consequences of disrupting the *FTS_1402* gene of the putative *protein glycosylation locus (pgl)*. Based on the homology to *Campylobacter* PglK flippase, FTS_1402 is predicted to play a role as a transporter of the nascent glycan across the inner membrane before its addition to target protein. Surprisingly, inactivation of the *FTS_1402* gene had a pleiotropic effect upon production of multiple surface glycoconjugates (e.g. glycoproteins, lipopolysaccharide, capsule/capsule-like complex), which all had preserved structures but were synthesized in smaller amounts when compared to the highly virulent parental strain. It resulted in unexpectedly marked attenuation of *FTS_1402* mutant with enhanced sensitivity to serum complement, which however, provided protection against subsequent systemic challenge with homologous wild-type FSC200 strain. This observation is of particular interest since a complete lack of surface glycoconjugates usually results in a failure to elicit protective immune response. Moreover, we provide evidence that a pilin glycoprotein PilA is modified with a single O-antigen unit of LPS in the *F. tularensis* FSC200 strain and its derived mutants.

S3-5
A phosphatidylinositol 3-kinase effector alters phagosomal trafficking to promote intracellular growth of *Francisella*

Hannah Ledvina[1], Katherine Kelly[2], Aria Eshraghi[2], Rachael Plemel[3], Snow Peterson[4], Brian Lee[5], Shaun Steele[6], Marlen Adler[2], Thomas Kawula[7], Alexey Merz[8], Shawn Skerrett[9], Jean Celli[6], Joseph Mougous[10]
[1]Department of Microbiology, University of Washington School of Medicine, Seattle, Washington, [2]Department of Microbiology, University of Washington School of Medicine, Seattle, [3]Department of Biochemistry, University of Washington School of Medicine, Seattle, [4]1Department of Microbiology, University of Washington School of Medicine, Seattle, [5]Division of Pulmonary, Critical Care and Sleep Medicine, Harborview Medical Center, Seattle, [6]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, [7]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, [8]Department of Biochemistry, University of Washington School of Medicine; Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, [9]Division of Pulmonary, Critical Care and Sleep Medicine, Harborview Medical Center, University of Washington, Seattle, [10]Department of Microbiology, University of Washington School of Medicine; Department of Biochemistry, University of Washington School of Medicine; Howard Hughes Medical Institute, University of Washington, Seattle

The success of intracellular bacteria relies on their ability to establish and maintain a permissive niche. By mechanisms that have remained undefined, a *Francisella* pathogenicity island-encoded secretion system allows bacteria in this genus to escape from phagosomes and replicate within the host cell cytoplasm. At this conference, I will present our discovery that a substrate of this secretion system, OpiA, represents a previously undescribed family of wortmannin-resistant bacterial phosphatidylinositide (PI) 3-kinase enzymes. Representatives of this family can be found in a wide range of intracellular pathogens, including *Rickettsia* and *Legionella* spp, as well as in many environmental bacteria. I will show that OpiA is recruited to endocytic membranes by binding PI(3)-phosphate (PI(3)P) and that it acts on the *Francisella*-containing phagosome to promote bacterial escape. Finally, I will present experiments demonstrating that arrest of endosomal maturation mitigates the phenotypic consequences of OpiA inactivation. In total, our findings suggest that Francisella, and likely other intracellular bacteria, override the finely-tuned dynamics of endosomal PI(3)P in order to delay phagosome maturation and promote intracellular survival and pathogenesis.

S3-6
Identifying virulence factors secreted by *Francisella tularensis* during infection

Peter Benziger[1], Erik Kopping[1], David Thanassi[2]
[1]*Stony Brook University, Stony Brook, New York*

Francisella tularensis is a successful pathogen in part because of its ability to invade a variety of host cells and replicate intracellularly following phagocytosis. While several virulence factors have been identified as critical for *Francisella* pathogenesis, how the bacteria manipulate the host cell to maintain their intracellular niche remains to be fully elucidated. To date, genes encoding both a type I secretion system (T1SS) and type VI secretion system (T6SS) have been identified in *F. tularensis* as potential mechanisms for delivering virulence factors to host cells. Recent evidence indicates that phagosomal escape is dependent on the T6SS; however, the repertoire of substrates secreted via the T6SS during infection is unclear. Additionally, our lab has identified TolC in *F. tularensis* as important for the bacteria's ability to delay host cell death. TolC is an outer membrane channel protein of the T1SS found across various Gram-negative bacteria and is associated with virulence. We found that the loss of TolC during infection leads to increased cytotoxicity and premature host cell death compared to the wild-type bacteria. In addition, the absence of TolC during an *in vivo* mouse infection model leads to decreased virulence. Therefore, we hypothesize that TolC functions in virulence protein secretion during the cytosolic stage of growth to delay host cell death. To address our hypothesis, we have employed bio-orthogonal non-canonical amino acid tagging (BONCAT) in combination with click chemistry to detect proteins secreted during infection through TolC. In brief, we have engineered *F. tularensis* with a tRNA synthase to incorporate chemically modified methionine during protein synthesis. A biotin-alkyne tag can then be covalently bound to the modified methionine, allowing for bacterial proteins secreted into the host cytosol to be detected by streptavidin. Identifying effectors secreted by *Francisella* will be critical in furthering our understanding of *F. tularensis* pathogenesis and revealing mechanisms by which it manipulates host cells responses.

S3-7
Hidden in plain erythrocyte

Joseph Horzempa[1], Stuart Cantlay[1], Deanna Schmitt[1]
[1]*West Liberty University, West Liberty, WV*

We have shown that *F. tularensis* invades and persists in erythrocytes. This invasion enhances resistance to antibiotics and enhances the ability of this bacterium to colonize ticks, important disease vectors. Investigations show that a subset of bacterial genes induced in the presence of erythrocytes are important for invasion of these host cells. Moreover, structural proteins of the the bacterial type VI secretion system (T6SS) are required for invasion, suggesting that this apparatus secretes effector molecules into erythroctyes to mediate invasion. We are currently investigating which bacterial genes induced in the presence of erythrocytes and T6SS effector proteins are required for invasion of these host cells. Regarding host factors required for invasion, data suggest that either the erythrocyte surface protein, Band 3, or the cytoskeletal protein, spectrin, are required for invasion. We are currently investigating whether Band 3, spectrin, or both host molecules are required for red blood cell invasion by *F. tularensis.*

Session 4

Bacteriology and Gene Regulation

S4-1
The role of the dynamic Type VI Secretion System in *Francisella* virulence

Maj Brodmann[1], Roland Dreier[1], Petr Broz[2], Marek Basler[1]
[1]*Biozentrum, University of Basel, Basel, Switzerland,* [2]*University of Lausanne, Lausanne, Switzerland*

The model organism *Francisella tularensis* subspecies *novicida* (*F. novicida*) is frequently used to study the fatal zoonotic disease tularemia and *Francisella* virulence. The advantage of *F. novicida* is that it harbors only one *Francisella* Pathogenicity Island (FPI). The FPI is critical for phagosomal escape and thus *Francisella* virulence and encodes a non-canonical Type VI Secretion System (T6SS). Hallmark of the T6SS is the dynamic assembly of a long cytosolic sheath and its rapid contraction to deliver effector proteins across target cell membranes of both bacterial and eukaryotic cells. Intriguingly, the FPI lacks several conserved components including the ATPase ClpV, which is essential for disassembly of contracted sheaths and T6SS dynamics in canonical T6SS. On the other hand, the FPI encodes many *Francisella* specific genes with unknown function. This unique gene composition raised our interest in understanding the assembly and dynamics of *Francisella* T6SS. Here we used live-cell fluorescence microscopy to show that in *F. novicida* the T6SS dynamics of assembly and disassembly on agar pads and in live macrophages are similar to dynamics of canonical T6SS. Strikingly, *Francisella* T6SS is preferentially located at the bacterial poles. General-purpose unfoldase ClpB co-localizes with the contracted T6SS sheath and is required for its disassembly. We analyzed T6SS dynamics in in-frame deletion mutants lacking genes with unknown function. Thereby, we identified components necessary for assembly (*iglF*, *iglG*, *iglI* and *iglJ*) and two potential effectors (*pdpC* and *pdpD*), which are not required for T6SS dynamics but bacteria lacking these two genes are unable to escape the phagosome, activate AIM2 inflammasome or cause disease in mice.

Expression plasmids are difficult to use in *F. novicida*, as they are prone to spontaneous deletions or impeded by restriction-modification systems. To overcome this, we constructed two mobilizable and tunable expression plasmids pJB1 and pJB2 with different ranges of maximum expression levels. Both plasmids contain a tetracycline inducible promoter to control expression levels and *oriT* for RP4 mediated mobilization. pJB1 and pJB2 were used to restore T6SS activity in *iglF*, *iglI* and *iglC* deletion mutants, excluding the possibility of polar effects on downstream genes. High efficiency of transfer and tunable expression of these plasmids will facilitate future studies in *F. novicida*.

S4-2
Plan B: Identifying a Backup Ribosome Rescue System in *Francisella tularensis*

Tyler Goralski[1], Girish Kirimanjeswara[1], Kenneth Keiler[1]
[1]*The Pennsylvania State University, State College, PA*

If a bacterial ribosome reaches the 3' end of the mRNA with no stop codon, the ribosome becomes stalled in a non-stop translation complex. This is detrimental to the bacteria, which can no longer use the stalled ribosome to aid in the translation of more protein. The ability to rescue ribosomes stalled at the ends of mRNAs is essential for efficient translation, and bacteria lacking ribosome rescue die. The primary pathway for ribosome rescue is *trans*-translation, the genes for which are conserved in >99% of sequenced bacteria. Backup systems, such as ArfA or ArfB have been identified in some bacteria, which can rescue ribosomes in the absence of sufficient *trans*-translation activity. We have previously shown that small molecule inhibitors that target all known ribosome rescue pathways can inhibit growth of *Francisella tularensis*. Interestingly, it has been shown that *trans*-translation is not critical for survival of *F. tularensis*, suggesting that this bacterium has an additional backup system, although there are no homologues to ArfA or ArfB. To identify potential backup ribosome rescue pathways, we used transposon mutagenesis followed by deep sequencing (Tn-Seq). We have identified and characterized a novel backup ribosome rescue system, alternate ribosome rescue factor T (ArfT), in *F. tularensis*, which has an amino acid sequence different from ArfA and ArfB. ArfT was found to be essential for survival in bacteria lacking *trans*-translation. Furthermore, ArfT has a distinct mechanism of action, interacting with release factor 1 (RF1), which binds to the stalled ribosome and hydrolyzes the nascent peptidyl-tRNA, whereas ArfA recruits peptide chain release factor 2 (RF2). Overexpression of ArfT rescued the growth defect observed in *F. tularensis* Live Vaccine Strain (LVS) lacking *trans*-translation, suggesting that ArfT can rescue ribosomes in the absence of *trans*-translation. Additionally, small molecule inhibitors of ribosome rescue arrest growth of *F. tularensis* LVS lacking *trans*-translation at equivalent concentrations as wild type LVS, indicating that these inhibitors also target ArfT. The identification and characterization of an alternate rescue factor in *F. tularensis* has allowed fuller insight into the physiology of ribosome rescue, and has elucidated another target for ribosome rescue inhibitors.

S4-3
Metabolic control of outer membrane vesicle and tube formation by *Francisella novicida*

Maheen Rashid[1], Vinaya Sampath[1], **David Thanassi**[1]
[1]*Stony Brook University, Stony Brook, New York*

Francisella novicida produces outer membrane vesicles as well as unusual tube-shaped structures during growth in specific media and during infection of macropahges. These outer membrane vesicles and tubes (OMV/T) likely act as a secretory system for host-pathogen interactions as their cargo includes known virulence factors. However, little is understood about the genetic and environmental factors that regulate OMV/T formation by *Francisella.* Using a genetic screen, we recently identified *F.novicida* gene products that regulate OMV/T production. Hypo-vesiculating mutants isolated from our screen include mutations in fumarate hydratase (*fumA*) and transketolase (*tktA*) – genes that have roles in central carbon metabolism. Analysis of these mutants identified amino acid depletion as a key nutrient signal for increased vesicle and tube formation. Our screen also identified a number of hyper-vesiculating mutants. Consistent with a role for amino acids in regulation of OMV/T biogenesis, these mutants included genes involved in the stringent starvation response, including the known virulence regulators MglA/SspA and RelA/SpoT homologs. The bacterial stringent starvation response is known to coordinate many metabolic pathways. Elucidating the roles of these genes will provide further insight into the links between amino acid starvation, carbon metabolism, virulence regulation and OMV/T formation by *Francisella.* We hypothesize that *Francisella*senses nutrient starvation as a cue to activate virulence regulators that in turn control OMV/T production. Ultimately, the findings from our study will provide a framework for understanding this phenomenon in the fully virulent pathogen, *F. tularenisis,*which also produces OMV/T in response to amino acid starvation.

S4-4
Structural and functional analysis of a putative lysine decarboxylase found in *Francisella* spp.

Jan Felix[1], Claire Siebert[2], Irina Gutsche[1], **Patricia Renesto**[3]
[1]IBS UGA, CNRS, CEA, Grenoble, France, [2]TIMC-IMAG UMR 5525 - UGA CNRS, CHU Grenoble Alpes, Grenoble, France, [3]TIMC-IMAG UMR5525, CNRS-UGA, Grenoble, France

Polyamines are ubiquitous and essential aliphatic polycations found in almost all living organisms. Their biosynthesis, which contribute to the optimized virulence or survival of several important bacterial pathogens, is dependent on the activity of decarboxylases of basic amino acids i.e. lysine, arginine and ornithine (LAO-DC). Bioinformatic analysis of the genomes of bacteria from the *Francisella* genus evidenced the presence of only one gene encoding a protein homologous to LAO-DC. *F. tularensis* which is responsible for the re-emerging disease tularemia, is endowed with an extreme acid resistance system of unknown genetic basis accounting for waterborne outbreaks and foodborne illness. Using *F.novicida* as model, we thus investigated whether this putative lysine decarboxylase (LDC-F) could help bacteria to survive environmental insults including low pH exposure.

Negative stain EM observations showed that, similarly to the constitutive and inducible *E. coli* LDCs, the *Francisella* protein assembles as decamer at pH7 and dissociates at higher pH. In addition, the crystallization trials achieved with the oligomeric protein were successful and allowed to obtain a crystal structure of the decamer at around 3.5 Å resolution.

To evaluate the functional role of LDC-F, we analyzed the phenotype of a *F. novicida*-Δ*ldcF* mutant. The phenotypic traits examined were related to the polyamine activity reported so far, such as the bacterial fitness under acidic and basic conditions, oxidative stress, antibiotic resistance, and biofilm formation. From these *in vitro* experiments we obtained evidence that overnight incubation of bacteria at 4°C in a nutrient-poor medium (creek water) slowed down the exponential growth rate of *F. novicida*-Δ*ldcF* as compared to that of the wild-type strain. The lag phase delay to reach an OD_{600nm} of 1 (1.3×10^9 bacteria/ml) remains stable for incubation in a low-nutrient environment ranging from 12h to several weeks. We also observed that *F. novicida*-Δ*ldcF* was less resistant to H_2O_2. In agreement with these results, *F. novicida*-Δ*ldcF* was found to be more sensitive to the killing activity of macrophages that couples exposure to both acidic and oxidative stress.

A better understanding of the way by which LDC-F contributes to bacterial virulence or persistence in environment should provide new targets for prevention or treatment of tularemia.

S4-5
Understanding the Mechanisms of Oxidative Stress Responses and Virulence of *Francisella tularensis* LVS

Meenakshi Malik[1], Zhuo Ma[1], Madeline Worden[1], Kayla King[1], Chandra Bakshi[2]
[1]Albany College of Pharmacy and Health Sciences, Albany, New York, [2]New York Medical College, Valhalla, NY

Francisella tularensis (*Ft*) is an important Gram-negative facultative intracellular human pathogen responsible for causing tularemia. It is also classified as a category A agent by the CDC based on its potential use as a bioterror agent. The molecular basis for the high infectivity and virulence of *Ft* is not well understood. However, the pathogenicity of *Ft* is mainly dependent on its ability to persist and replicate in phagocytic cells. Multiple antioxidant enzymes that are important defense factors against oxidative stress have been identified and implicated in pathogenesis of tularemia. Our previous study has demonstrated that some of these antioxidant enzymes are regulated by a LysR family transcriptional regulator, OxyR. In the present study, we further investigate the roles of pp(p)Gpp; the stringent response molecules produced by RelA and SpoT proteins, in the oxidative stress response and virulence of *Ft*. We generated a *relA* gene deletion mutant (Δ*relA*), a *relA*/*spoT* double gene mutant (Δ*relA*/*spoT*) and corresponding transcomplements of *Ft* LVS. These mutants were characterized for their sensitivity towards oxidants, ability to survive in macrophages and for their virulence in mice. As compared with wild type *Ft* LVS, the *ΔrelA*/*spoT* mutant grew slowly at high temperatures. The *ΔrelA*/*spoT* double mutant, which is unable to produce pp(p)Gpp, showed strong sensitivity towards oxidants; defective replication and survival in macrophages, and attenuated virulence in mice. Further studies employing RNA-seq and qRT-PCR revealed the global effect of pp(p)Gpp on the expression of antioxidant enzyme genes, multiple heat shock protein genes; and genes located on Francisella Pathogenecity Island. These results demonstrate that RelA/SpoT of *Ft* play an important role in providing resistance against oxidative stress and thereby facilitate intramacrophage survival and virulence in mice.

Poster Session #2

P2-01
Natural history of pneumonic tularemia in outbred New Zealand White Rabbits

Jennifer Bowling[1], Elizabeth Stinson[1], Katherine Willett - O'Malley[1], Karsten Hazlett[2], Eileen Barry[3], Doug Reed[1]
[1]University of Pittsburgh, Pittsburgh, PA, [2]Albany Medical College, Albany, [3]University of Maryland at Baltimore, Baltimore, MD

We have previously reestablished the New Zealand White (NZW) rabbit as a model for pneumonic tularemia in humans. Clinical signs of disease include fever, weight loss, dehydration, anorexia, behavioral changes and changes in appearance; naïve rabbits succumb within 4-7 days post-infection (dpi). Radiographs indicate the presence of a severe bacterial pneumonia. To further characterize this model, we examined the natural history of pneumonic tularemia in rabbits. Rabbits were challenged via aerosol with $100LD_{50}$ of Ft SCHUS4 and euthanized at 24-hour time points to collect samples for analysis. Co-incident with the onset of fever (2.5-3 dpi) we see an increase in erythrocyte sedimentation rate (ESR), thrombocytopenia, and lymphopenia (66%) at 3 dpi. Monocyte and granulocyte populations did not significantly change in number over the course of the disease. Albumin decreases and BUN increases beginning on 3 dpi correlate with anorexia and dehydration. On 4 dpi, alanine transaminase and total bilirubin increase while bile acids decrease, suggesting liver injury. Lung sections taken at necropsy had indications of inflammation in the lungs as early as 1 dpi which increased rapidly over the time course of the disease. Similarly, TUNEL staining found evidence of DNA fragmentation, indicative of apoptosis, as early as 1 dpi and increasing progressively through 5 dpi. Bacterial titers in bronchoalveolar lavage and lung digests were positive on 1 dpi and increased 100-fold by 3 dpi. Although bacteremia levels remained low throughout the course of the disease, bacteria were found in the spleen of 1 of 3 rabbits necropsied on 1 dpi, suggesting dissemination can occur early in the course of the disease. In rabbits that succumbed to the disease, bacterial titers were highest in the lung and spleen but were also found in the liver, kidney, intestines, and lymph node. These results and additional analyses that are ongoing will be discussed.

P2-02

Production of IFN-γ by multiple splenic leukocytes during innate immune responses to *Francisella tularensis* LVS depends on MyD88, but not TLR2, TLR4, or TLR9

Amy Poupore[1], Karen Elkins[1], **Roberto De Pascalis**[1]
[1]*CBER/FDA, Silver Spring*

In infections with *Francisella tularensis*, early production of IFN-γ is a key innate immune mechanism that limits initial bacterial replication until adaptive immune responses develop. Previously we demonstrated that cell types responsible for innate IFN-γ production are highly diversified and include not only natural killer (NK) and T cells but also neutrophils, dendritic cells (DCs), NK T cells, and hybrid NK DCs. However, DC production of IFN-γ is controversial. Here, we directly demonstrated production of IFN-γ by DCs from LVS-infected wild type C57BL/6 or RAG mice. These studies also characterized hybrid NK DCs, which contribute significantly to IFN-γ production. We demonstrated that the number of conventional NK cells ($NK1.1^+$ $MHCII^-$ $CD11c^-$) producing IFN-γ, which represented about 50% of non-B/T IFN-γ-producing cells, peaked at day 4 after LVS infection in a pattern similar to that of hybrid NK DCs, mostly $NK1.1^+$ $MHCII^+$ $CD11c^+$. In contrast, numbers of conventional DCs ($NK1.1^-$ $MHCII^+$ $CD11c^+$ $B220^-$ $CD11b^+$ $Gr1^-$) producing IFN-γ increased progressively over the course of 8 days of LVS infection. Similar IFN-γ production patterns were observed in cells derived from LVS-infected TLR2, TLR4 and TLR2x9 KO mice, but not from MyD88 KO mice. To further confirm IFN-γ production by infected cells, non-B/T cells from naïve and LVS-infected mice were sorted and analyzed for gene expression. Quantification of LVS by PCR revealed the presence of *Ft* DNA not only in macrophages, but also in DCs and neutrophils. Consistent with increased bacterial burdens measured by plate count, LVS DNA content was 2-3 fold and 300-3000 fold higher in DCs and neutrophils from TLR2 KO and MyD88 KO mice, respectively. Highly purified DCs and neutrophils from both WT and TLR2 KO mice clearly expressed IFN-γ. Also of note, in contrast to IFN-γ, the expression of IL1a, IL-12b, and TNF-α was not affected in DCs and neutrophils from LVS-infected MyD88 KO mice. Taken together, these studies confirm the primary role of IFN-γ and MyD88 in mediating the innate immune response and the importance of understanding the final steps of the pathway that lead to IFN-γ production. TLR2 KO mice can circumvent the lack of TLR2-mediated pathway activation and produce comparable amount of IFN-γ as WT mice, and as a result LVS infection is only modestly compromised.

P2-03
Establishment of an *in vitro* approach to study interactions between *Francisella*, alveolar macrophages, and immune T cells

Helen Brook[1], **Varunika Bhargava**[1], Sherry Kurtz[1], Karen Elkins[1]
[1]*CBER/FDA, Silver Spring, MD*

Francisella tularensis infects mammals by a variety of different routes, resulting in tularemia with different manifestations that reflect the bacterium's route of entry. *F. tularensis* invades and replicates within host cells, especially macrophages, but macrophages in tissues vary widely in phenotype and functions. Similarly, tissue-resident lymphocytes embedded within different tissues vary in functional capacities. Most studies to date of *Francisella*-macrophage interactions have used convenient sources such as bone marrow-derived macrophages from rodents or peripheral blood monocytes from people, but alveolar macrophages are the first cells attacked during respiratory tularemia. Here we initiated approaches to study the interactions of alveolar macrophages with immune lymphocytes, including lung lymphocytes. We obtained mouse primary alveolar macrophages from bronchial lavage of C57BL/6J mice, and optimized conditions to study *in vitro* infections with *Francisella.* Alveolar macrophage monolayers were readily infected *in vitro* with the Live Vaccine Strain (LVS) of *F. tularensis*, and LVS replicated exponentially within alveolar macrophages over the course of 2 – 3 days. Co-culture of naïve splenocytes with LVS-infected alveolar macrophages had little impact on bacterial replication. In contrast, co-culture of LVS-immune splenocytes with LVS-infected alveolar macrophages greatly reduced bacterial replication. Control of LVS growth within alveolar macrophages by LVS-immune splenocytes was associated with production of much less Interferon-gamma (IFN-γ) than control within bone marrow-derived macrophages. Current experiments are therefore determining the role of IFN-γ and other mediators in bacterial growth control for each type of macrophages. This work therefore lays the groundwork to study the interactions of *Francisella*-infected lung macrophages and lymphocytes in order to determine mechanisms of intramacrophage bacterial growth control in detail.

P2-04
Quality Assurance in Tularemia Diagnostics in Europe

Daniela Jacob[1], Roland Grunow[1], Appelt Sandra[1], Anna Rohleder[1]
[1]*Robert Koch Institute, Berlin, Germany*

Laboratory diagnostics on tularemia imposes high demands, especially on the conducting institution. Agent detection, especially the challenge to cultivate *Francisella (F.) tularensis*, requires appropriate sample material, as well as special culture media and incubation conditions. For confirmation, specific PCR methods and/or MALDI-ToF analyses are required. Without a tentative clinical diagnosis, *F. tularensis* can easily be overlooked. Laboratory diagnosis, including bacterial detection and serology of *Francisella*, is often done by in-house-tests underlying special validation. To date, there are hardly any accredited laboratory diagnostics in human medicine available for tularemia in Germany. Necessary validation measures have to be adapted to the requirements and possibilities of a rare disease - internal as well as external quality assurance measures are required.
In this context, the EU-funded Joint Action EMERGE (Decision No 082/2013/EU) offers a possibility for quality assurance of agent detection. Including further highly pathogenic bacterial (and viral) agents, proficiency tests are offered, which have been organized by the Robert Koch Institute over eight years now. Together with the results gained from the former projects EQADeBa and QUANDHIP, it can be deduced that, for this kind of diagnostics, European BSL3 labs are operating on a very high level in terms of quality and skills. For the diagnostic of *F. tularensis*, we can assume that especially the subspecies differentiation should be further improved.
Beneath microbiological, molecular biological, immunological and other methods for agent detection, serological analyses for antibody detection should be included in external quality exercises. While providing the results of these exercises, the most common problems of diagnostic approaches will be discussed.

P2-05
Genetic requirements for the intracellular growth of *Francisella tularensis*

Kathryn Ramsey[1], Thomas Tallo[2], Simon Dove[2]
[1]University of Rhode Island, Kingston, RI, [2]Boston Children's Hospital, Harvard Medical School, Boston, MA

Francisella tularensis is a facultative intracellular pathogen that is considered a potential bioweapon. The mechanisms underpinning *F. tularensis* virulence are still incompletely understood. To date, there has not been an exhaustive identification of genes important for survival of *F. tularensis* either *in vitro* or in macrophage, which is a niche critical for the ability of *F. tularensis* to cause disease. In order to address this gap in knowledge, we performed a genome-wide screen for genes in *F. tularensis* LVS essential for growth *in vitro* and for growth in macrophages using a highly saturated library of transposon mutants together with high-throughput sequencing (Tn-Seq). We created a transposon insertion library that contains a mariner transposon insertion in 75% of TA sites, corresponding to an insertion, on average, every twelve base pairs. Growth of this library *in vitro* and in macrophage allowed us to identify not only genes, but also domains of corresponding proteins, that are required for survival in these different conditions. Our findings indicate over a quarter of *F. tularensis* genes are critical for *in vitro* growth, and an additional approximately 140 genes are critical for intramacrophage growth. This work represents a comprehensive genome-wide identification of genetic elements required for the intracellular growth of *F. tularensis*.

P2-06
Identification and characterization of *rgbP*, a gene required for optimal growth of *Francisella tularensis* at mammalian physiological pH

David Williamson[1], Kalyan Dewan[1], Bhuvana Katkere[1], Rachel Markley[1], **Girish Kirimanjeswara**[1]
[1]*Penn State University, University Park, PA*

pH homeostasis is important to all living cells, as it is intimately related to protein function and proton motive force. In mammalian hosts, many potential growth niches are strictly regulated near the 'physiological' pH of 7.4, yet studies of bacterial pH homeostasis have focused on the more extreme ends of the pH range tolerated by model organisms (pH 5.5-9.0). The facultative intracellular pathogen *Francisella tularensis* fails to grow in media above pH ~7.4, and exhibits morphological and metabolic changes at pH 7.4 compared to its optimal growth pH of <6.8. We hypothesized that adaptive changes are required for *F. tularensis* to grow at pH 7.4, and that these changes would also be required for virulence in the mammalian host. In this study, we identify a gene coding for a hitherto uncharacterized hypothetical protein that is required for growth of *F. tularensis* at pH 7.3-7.4 (termed *rgbP*: *r*equired for *g*rowth at slightly *b*asic *p*H). Mutants lacking this gene exhibit a pH-dependent growth defect in media and in the cytosol of mammalian macrophages. The presence of wild-type *F. tularensis* in an infected cell does not restore growth of *ΔrgbP*, supporting a role intrinsic to the bacterial cell rather than an effect exerted on the surrounding environment. *ΔrgbP* is highly attenuated in mouse models of tularemia; compared to wild-type-infected mice, *ΔrgbP* –infected mice exhibit decreased mortality, weight loss, bacterial burden, and lower levels of multiple pro-inflammatory cytokines including IL-1β and IFN-γ. *ΔrgbP* displays an altered metabolic profile compared to the wild-type, including constitutively lower levels of the electron carrier $NADP^+$, constitutively higher levels of succinate and fumarate, and higher levels of lactate and acetate at pH 7.4. The mutant also displayed an aberrent morpholgy consistent with lack of adaptive changes in the membrane when grown at pH 7.4. This study demonstrates that bacterial strategies to maintain pH homeostasis are important even at the near-neutral physiological pH of mammalian cells.

P2-07
Insights into the moonlighting of *Francisella tularensis* glyceraldehyde-3-phosphate dehydrogenase

Monika Kopeckova[1], Ivona Pavkova[1], Helena Rehulkova[1], Pavel Rehulka[1]
[1]*Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic*

The enzyme of glycolytic pathway – glyceraldehyde-3-phosphate dehydrogenase (GAPDH) - is well-known for its numerous non-metabolic functions in both eukaryotic and prokaryotic cells. Such additional activities require distinct subcellular distribution and may play important roles in the virulence characteristics of a number of human pathogens. The results of our previously proteome analysis indicated that GapA, the GAPDH homologue of the virulent strain of *Francisella tularensis* subsp. *holarctica* FSC200, could act in a similar way. We thus performed further functional studies on this enzyme to investigate its phenotype and subcellular localization. The deletion of the *gapA* gene resulted in a viable mutant strain, characterized by reduced virulence in mice, defective replication inside macrophages, and its ability to induce a protective immune response against systemic challenge with parental wild-type strain. The protein has been demonstrated in multiple localization sites including the cytosol, cell surface and the culture medium. Furthermore the recombinant GapA was shown to bind to host extracellular serum proteins like plasminogen, fibrinogen, and fibronectin. In particular the extracytosolic localization of GapA and its ability to bind to host proteins provide convincing evidence of its additional non-enzymatic functions. The ongoing studies are thus focused on the elucidation of interaction partners both in bacteria and host cells to specify more precisely non-enzymatic roles of *F. tularensis* GapA. For this purposes we decided to apply the affinity purification of tagged GapA followed by SILAC-based quantitative mass spectrometry (AP-MS) as this technique enables confident discrimination between specific and non-specific interactors. In the first preliminary experiments several bacterial proteins as potential interaction partners could be identified. We will next confirm these interactions by additional methods. To identify host proteins targeted by *F. tularensis* GapA, the tagged GapA will be expressed in SILAC labeled host cell line followed by AP-MS.

P2-08
Modulation of host cell apoptosis by *Francisella tularensis*

Erik Kopping, David Thanassi
[1]*Stony Brook University, Stony Brook, New York*

The ability to suppress the early innate immune response is a hallmark of *Francisella tularensis* infection and central to virulence in the mammalian host. As an intracellular pathogen, *F. tularensis* must evade host defenses including anti-microbial peptides, reactive oxygen/nitrogen species, and innate immune signaling pathways. These early events are key to establishing the host cell cytosol as a protected replicative niche. Although the molecular details governing this early host cell modulation remain to be elucidated, work from our lab and others has established that *F. tularensis* actively suppresses apoptotic responses to preserve host cell viability. Previous work from our lab revealed that *F. tularensis* uses its outer membrane channel protein TolC to delay the intrinsic pathway of apoptosis in infected macrophages. TolC is a component of the type I secretion system (T1SS), which functions in the export of bacterial virulence factors. Yet, the mechanism by which *F. tularensis* delays the intrinsic pathway of apoptosis via TolC during infection remains unknown. To better understand this, we sought to identify what triggers apoptotic death in infected macrophages during infection with the *F. tularensis* Δ*tolC* mutant. Central to preserving cell viability is mitochondrial integrity. Disturbances in mitochondrial integrity, such as damage from redox stress, activate signaling cascades that permeabilize the organelle and trigger the intrinsic pathway of apoptosis. We found that treatment of cells with antioxidants such as glutathione (GSH) or n-acetylcysteine (NAC) block macrophage apoptotic responses to the Δ*tolC* mutant. Furthermore, treatment of cells with specific inhibitors to either the NADPH oxidase (apocynin) or mitochondrial superoxide (MitoTEMPO) also block macrophage apoptosis in response to the Δ*tolC* mutant. Using flow cytometry-based assays, we observed that infection of macrophages with the Δ*tolC* mutant leads to increased generation of cellular ROS species. Of note, generation of mitochondrial superoxide was elevated in response to the Δ*tolC* mutant, which may contribute to mitochondrial dysfunction and activation of the intrinsic pathway of apoptosis. We are currently investigating if *F. tularensis* delivers antioxidant enzymes into the host cell cytosol in a TolC-dependent manner to suppress host redox stress and delay the induction of apoptosis.

P2-09
Development of a non-human primate *in vitro* growth inhibition assay to predict vaccine efficacy against *F. tularensis*

Sherry Kurtz[1], Herbert Gelhaus[2], Karen Elkins[1]
[1]CBER/FDA, Silver Spring, [2]MRIGlobal, Kansas City, MO

Because new vaccines are being developed for the prevention of tularemia, establishing new strategies to study vaccine efficacy and to determine correlates of immunity is important. To this end, our laboratory used *Francisella*-immune inbred mice and rats to develop an *in vitro* co-culture system that itself is a functional correlate of vaccine-induced protection. This assay incorporates a macrophage monolayer derived from bone marrow (BMM) that is infected with *Francisella* LVS, the live attenuated human vaccine against tularemia, which serves as a surrogate for virulent *Francisella*. Infected monocytes were then co-cultured with immune lymphocytes from vaccinated animals. Intracellular *Francisella* replicates exponentially over several days in BMM alone or BMM with naïve immune cells, but growth is reduced in the BMM co-cultured with immune lymphocytes primed by a protective vaccine in patterns that reflect the degree of vaccine efficacy. Here, we advanced these studies to study cells from non-human primates, an animal model that may more closely recapitulate human disease. Because NHPs are outbred and heterogeneous, the approach was modified to co-culture MHC-matched macrophages and lymphocytes from the same animal. We isolated circulating monocytes from peripheral blood lymphocytes (PBLs) by adherence and used these as the macrophage source for the assay. We optimized the ratio of infection of *Francisella*:monocytes to achieve maximal bacterial growth, and demonstrated that LVS as well as other attenuated *Francisella* vaccine candidates replicated exponentially in NHP monocytes over three days. When re-combined with infected monocytes, PBLs from naïve animals did not alter the growth of the bacteria in the monocytes, and IFN-g or nitric oxide in co-culture supernatants will be measured. We are currently evaluating PBLs from LVS-vaccinated NHPs to determine the capability of these cells to restrict intracellular LVS growth. In sum, we have adapted the format of the original rodent co-culture assay for use with NHP cells, and we are now prepared to test the assay as a functional correlate of protection in *Francisella*-vaccinated NHPs. Future studies will further evaluate biomarker production in co-culture assays as additional correlates.

P2-10
Human tularemia associated with exposure to domestic dogs — United States, 2006–2016

Natalie Kwit[1], Amy Schwartz[2], Kiersten Kugeler[2], Paul Mead[2], Christina Nelson[2]
[1]*Vermont Department of Health, Burlington,* [2]*CDC Division of Vector-Borne Diseases, Fort Collins, CO*

Background: Human infection with *Francisella tularensis* via cat bite is well documented; however, the role of dogs in transmitting infection is much less understood. The objective of this study was to characterize the scope and patterns of human tularemia cases with exposures linked to domestic dogs to improve awareness and targeting of prevention methods.

Methods: We reviewed available confirmed and probable human tularemia case investigation records supplemental to national surveillance data from 2006–2016 and extracted those with the following indicators: a pet dog that was recently ill, died, or brought home dead animals, or written comments that provided other evidence of dog-related exposure to *F. tularensis*. Two independent reviewers further evaluated the records in detail to select those demonstrating probable or possible dog-related *F. tularensis* exposure based upon strength of available evidence.

Results: Among 735 tularemia case investigation records reviewed, 24 (3.3%) were identified as dog-related. Median age of patients was 51 years (range: 1–82); 54% were female compared with 32% of all reported cases. Two-thirds (67%) of cases were ulceroglandular/glandular in presentation followed by pneumonic (13%) and oropharyngeal (13%). Among 20 patients with known clinical outcomes, 18 (75%) recovered from illness and two (8%) died. Illness was attributed to three main exposure types: direct contact via dog bites, scratches, or face snuggling/licking (n = 12; 50%), contact with dead animals retrieved by domestic dogs (n = 8; 33%), and dogs bringing infected ticks into contact with humans (n = 4; 17%). Four (17%) case records indicated exposure to ill dogs, but only one dog was evaluated for laboratory evidence of exposure to *F. tularensis*.

Conclusions: Although uncommon, dog-related tularemia infections highlight specific modifiable risk factors for pet owners who should be educated to use appropriate tick-control methods for dogs, take precautions in disposal of animal carcasses retrieved by dogs, exercise caution in direct facial contact with dogs who hunt or roam, and seek veterinary care for ill pets. Public health officials should be alert to the potential for dogs as a source of human infection and encourage veterinary evaluation of dogs linked to human cases.

P2-11
Vaccine-mediated mechanisms controlling *Francisella tularensis* SCHU S4 in rat mononuclear cells

Helena Lindgren[1], Kjell Eneslätt[1], Igor Golovliov[1], Herbert Gelhaus[2], Anders Sjöst-edt[1]
[1]Umeå University, UMEÅ, Sweden, [2]MRIGlobal, Kansas City, MO

Francisella tularensis (*F. tularensis*) is a highly virulent intracellular bacterium causing the severe disease tularemia in many mammalian species. The *F. tularensis* live vaccine strain (LVS) is a human vaccine strain, however, it does not confer efficacious protection against aerosol infection and is not licensed for public use. Therefore, there is a need for development of more efficacious *Francisella* vaccines. We previously identified that a Δ*clpB* mutant of SCHU S4 conferred superior efficacy in a mouse model compared to LVS and the double mutant *ΔclpB/ΔwbtC*. In contrast to mice, which are highly susceptible to virulent strains of *F. tularensis*, rat exhibit similar susceptibility as humans and may therefore be a better model than mice. In the present study, the T-cell mediated immunity against *F. tularensis* in rats was characterized using a co-culture system, based on *in vitro* SCHU S4-infected bone marrow-derived macrophages and splenocytes from naïve or LVS-, Δ*clpB*- or *ΔclpB/ΔwbtC*-immunized Fischer 344 rats. The results showed that co-cultures with splenocytes from *ΔclpB*-, LVS- or *ΔclpB/ΔwbtC*-immune rats and *F. tularensis*-infected BMDM elicited a complex immune response resulting in cytokine secretion, nitric oxide production, and efficient control of the intracellular growth of the highly virulent strain SCHU S4. However, there were qualitative differences among the groups and addition of Δ*clpB*-immune splenocytes elicited a slightly better control of bacterial growth than did LVS, whereas addition of Δ*clpB/wbtC*-immune splenocytes was the least effective. Concomitantly, lower levels of IFN-γ, TNF-α, fractalkine, IL-2 and nitrite were present in the co-cultures with *ΔclpB/ΔwbtC* splenocytes than in those with splenocytes from LVS- or Δ*clpB*-immunized rats. Nitric oxide appeared to be a correlate of protection since inhibition of nitric oxide production completely reversed the growth inhibition of SCHU S4. Overall the results support that the co-culture assay is a suitable model to identify correlates of protection against *F. tularensis.*

P2-12
First imported case of tularemia in Portugal

Isabel Lopes de Carvalho[1], Paula Nascimento[2], Maria Núncio[3], Miguel Toscano Rico[4]
[1]National Institute of Health, Infectious Diseases Department, Águas de Moura, Portugal, [2]Internal Medicine Service 4, Centro Hospitalar de Lisboa Central- Santa Marta Hospital, Lisboa, Portugal, [3]Infectious Diseases Department, National Institute of Health, Lisboa, Portugal, [4]Internal Medicine Service, Centro Hospitalar de Lisboa Central- Santa Marta Hospital, Lisboa, Portugal

Background: Tularemia is a zoonotic disease caused by *Francisella tularensis*. The transmission to humans occurs through direct skin or mucosal contact of infected animals, by inhalation of contaminated food or water. Although is considered an unusual disease recent outbreaks of tularemia have occurred in several European countries (Spain, France, Scandinavia, the Balkans and Hungary) and sporadic cases (Austria, Italy and the United Kingdom). In Portugal, the bacterium was detected first in the blood of an asymptomatic man and in a *Dermacentor reticulatus* tick by molecular methods.

Case Report:

The authors present the case of a 47-year-old man who walked in countryside on the island of Bornholm, Denmark, during the summer period. Three days later, fever, myalgias and adynamia began. On returning to Portugal, the 8th day of fever was observed with an erythematous macular rash on the cutaneous skin, with no palmoplantar involvement and an erythematous left infra supraclavicular lesion with a necrotic plaque, suggestive of eschar of inoculation. The presence of left lateral and left axillary adenopathy's was also evident.

Clinically the presentation suggested a zoonosis, very suggestive of Boutonneuse fever, for which doxycycline empirically was initiated, while awaiting serology results. However, the serologies for *Rickettsia spp.* and *Borrelia burgdorferi* s.l. were negative. Contextualizing the epidemiology and loco regional adenopathy conglomerate, the hypothesis of tularemia, in ulceroglandular form, later confirmed by serology (agglutination method and ELISA) was considered. Initially did doxycycline (4 days), later replaced by streptomycin 1g IM 12/12 (10 days). Despite the resolution of fever and rash, clinical improvement was not observed. The left axillary cervical and axillary adenopathy's increased in number and size, with the largest spontaneously being suppurated. For this reason, a ganglion was excised, with documentation of *F. tularensis* subsp. *holarctica* by bacterial growth in culture medium and by molecular methods. The clinical outcome was interpreted as therapeutic failure, and ciprofloxacin 500mg 12 / 12h (28 days) and adjuvant ganglionic drainage were started with clinical improvement.

P2-13
Role of Zinc in *Francisella* gene regulation

Barbara Mann[1], Brett Moreau[1]
[1]*University of Virginia, Charlottesville, VA*

Zinc is an essential nutrient for all cells, and one mechanism host cells use to control bacterial growth is to limit pathogen access to zinc. As *Francisella* grows effectively intracellularly, it must have efficient mechanisms of acquiring zinc from host cells, but these mechanisms have not been well characterized. Many bacteria use a zinc-responsive transcriptional regulator, Zur, to control expression of genes required for zinc uptake. To identify mechanisms of zinc uptake in *Francisella novicida* U112, RNA sequencing (RNASeq) was performed on wild-type and putative *zur* mutant bacteria. Only three genes were confirmed by quantitative RT-PCR as directly regulated by Zur and zinc limitation. One of these genes, FTN_0879, is predicted to encode a protein with similarity to the *zupT* family of zinc transporters, which are not typically regulated by Zur. While a *znuACB* operon encoding a zinc transporter was identified by bioinformatics in U112, expression of this operon was not controlled by Zur. Disruption of *zupT* but not *znuA* in U112 impaired growth under zinc limitation, suggesting that ZupT is the primary mechanism for zinc acquisition in these conditions. In the virulent *Francisella tularensis* subsp. *tularensis* Schu S4 strain, *zupT* is a pseudogene. Additional experiments revealed that ZnuACB is the primary mechanism of zinc uptake in Schu S4. Because so few Zur-regulated genes were identified, we sought to determine if there were Zur-independent mechanisms of response to zinc limitation. RNAseq analyses were performed on U112 and Schu S4 grown in the presence or absence of TPEN, a zinc-chelating agent. Nine genes were identified as differentially regulated in these conditions in U112, including the three Zur regulated genes. Twenty-two genes were differentially expressed in Schu S4. With the exception of the sole Zur-regulated gene, these Schu S4 genes did not overlap with U112. The majority of the Schu S4 genes were related to iron or oxidative stress. Together these results suggest that zinc-specific gene regulation mechanisms are limited in *Francisella* and also highlight differences between environmental and virulent strains.

P2-14

Development of immunoassays targeting the lipopolysaccharide of *Francisella tularensis* for rapid diagnosis of tularemia

Emily Hannah[1], Sujata G Pandit[1], Teerapat Nualnoi[1], Derrick Hau[1], Haley L DeMers[1], Kathryn Pflughoeft[1], Peter Thorkildson , Yasemin Ozsurekci[2], Marcellene Gates-Hollingsworth[1], David P AuCoin[1]

[1]University of Nevada, Reno, Reno, United States; [2]Hacettepe University Faculty of Medicine, Ankara, Turkey

Francisella tularensis is the causative agent of tularemia, a zoonotic bacterial infection that is often fatal if not treated correctly. Low infectious doses combined with a wide range of symptoms and a short incubation period makes timely diagnosis of tularemia difficult. Current diagnostic techniques rely on culturing of this fastidious, slow-growing bacterium from patient samples, a risky and unreliable process. Serological detection of *F. tularensis* is possible, however it may be too late in the course of infection before detection of host antibodies is possible. Both of these techniques require sophisticated equipment and highly trained laboratory personnel. In the event of a *F. tularensis* outbreak or exposure incident, a more efficient point-of-care (POC) diagnostic is needed. Using the *In Vivo* Microbial Antigen Discovery (InMAD) biomarker discovery platform the lipopolysaccharide (LPS) component of the bacterial outer leaflet was identified as a potential diagnostic biomarker that may be shed during infection. To develop an immunoassay for detection of LPS, CD-1 mice were immunized with purified *F. tularensis* LPS and ten hybridoma cell lines producing monoclonal antibodies highly reactive with F. tularensis LPS were isolated. These antibodies were confirmed to be reactive with *F. tularensis* LPS from both type A and type B strains. Antibodies were tested in an antigen-capture ELISA and lateral flow immunoassay (LFI) format to determine viable pairs. Selected pairs show high sensitivity when detecting purified *F. tularensis* LPS spiked into pooled human serum and urine. Antibody reactivity with LPS present in patient samples was also confirmed, suggesting that the assay may be useful for detection and quantification of low levels of LPS in clinical samples. As the ELISA requires time and laboratory expertise to perform, development began on a lateral flow immunoassay, a rapid diagnostic format that can be utilized at POC. A prototype LFI for diagnosis of tularemia has been developed and evaluated for sensitivity with spiked human samples. Assay optimization and evaluation of clinical samples is ongoing and will continue, with the end goal of developing a clinically relevant POC diagnostic that can be used in the event of an outbreak or widespread exposure to *F. tularensis*.

P2-15
Structure-function studies of *Francisella* YbeX

Gerard Nau[1], Joseph Horzempa[2], Ana Hernandez[3]
[1]Alpert School of Medicine at Brown University, Providence, Rhode Island, [2]West Liberty University, West Liberty, WV, WV, [3]Rhode Island Hospital, Providence

We have previously shown that *ybeX* is required for full virulence of *Francisella tularensis* and to limit stimulation of host immune cells. The function of YbeX is unclear, in part because early studies used different types of bacteria where phenotypes were not complemented. This is important because the 3' gene in Gram-negative organisms, apolipoprotein N-acyltransferase, is essential in nearly all other genera except for *Francisella*. The objective of this study was to investigate the relationship of YbeX structure and function as an approach to understand its contribution to pathogenesis. The crystal structure of *Escherichia coli* YbeX predicts formation of a homodimer. Using bimolecular fluorescence complementation (BiFC), we confirmed that *Francisella* YbeX forms homodimers. The protein is predicted to have an unstructured amino-terminal region (ATR), two CBS domains that mediate protein-protein interactions, and a carboxy-terminal CorC/HlyC domain. We found that only the CBS domains were required for dimerization in BiFC. All domains, however, were required for complementation of YbeX function in the Δ*ybeX* strain. These homodimerization results suggest two potential models for YbeX function. If homodimerization is constitutive, YbeX may function in a structural role within the bacterium. If homodimerization is a regulated event, YbeX may function as a cellular sensor or in a signal transduction pathway.

P2-16
CanSNPdb: A tool for efficient communication of *Francisella* taxa in the WGS era

Caroline Öhrman[1], David Sundell[1], Kerstin Myrtennäs[1], Dawn Birdsell[2]
[1]Swedish Defence Research Agency (FOI), Umeå, Sweden, [2]Northern Arizona University, Flagstaff, AZ

To keep pace with assigning unique official branch names to new lineages in the WGS era and to place an unknown *F. tularensis* isolates onto the existing global phylogenetic tree, we created a web platform that assists researchers to accomplish these objectives. Whole genome sequence (WGS) technology has eased the burden of identifying new phylogenetic lineages within *Francisella tularensis*. As a consequence, new lineages are being discovered at an unprecedented rate. This reality magnifies the need for efficient assignment of unique identifiers to every new lineage in a coordinated fashion. *F. tularensis* genome is highly clonal with very little evidence for recombination. For this, single nucleotide polymorphisms (SNPs) are highly stable genetic markers and are used to reconstruct *F. tularensis* phylogenetic framework. *F. tularensis* phylogenetic lineages (representing major groups, subgroups, and subclades) are defined by a single canonical SNP (canSNP) with a unique SNP coordinate. The indexing of SNP coordinates by aligning new sequences to a universal reference genome enables accurate inter-laboratory comparisons of identical SNP markers and cataloging informative SNPs in a universal global database.

The CanSNPdb web platform is being developed using the Django framework running on nginx and wugsi webserver together with sqlite3 for database handling. The current version of the *Francisella* canSNP phylogeny and the latest published canSNP index will be displayed on the frontpage. Users can contribute to the growing tree and give name to new branches by submitting their newly discovered canSNPs. Each newly discovered lineage will be given a unique index (official branch name) that is cataloged in the SNP database to avoid overlap with existing and future lineages. This branch name will be reserved and released upon publication. Genome metadata can be easily accessed by navigating through the site. Future revisions of the web platform will enable users to submit sequences or an assembled genome and display its position in the tree by simultaneous operation of CanSNPer. The end goal is to have the functionality that allows direct submission of new canSNPs for the supplied genome.

CanSNPdb web platform unifies naming of canSNPs and allows for efficient communication of *Francisella* taxa.

P2-17
Early cellular responses of germ free and specific pathogen free mice to *Francisella tularensis* infection

Lenka Plzakova[1], Zuzana Krocova[1], Klara Kubelkova[1], Ales Macela[1]
[1]*University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove, Czech Republic*

Francisella tularensis is a small, facultative intracellular Gram-negative coccobacillus, which caused tularemia in humans and in animals. *Francisella* spp. infect and proliferate inside phagocytic cell types, non-phagocytic hepatocytes, epithelial cells, and B cell lines both in *in vitro* and *in vivo* experimental systems. Information available about immune response to *Francisella* spp. come mostly from animal model studies. Our experiments have shown different pattern of cellular behavior after *F. tularensis* subsp. *holarctica* strain LVS FSC155 infection of germ free (GF) mice in comparison with SPF mice associated with. The mammalian intestine is colonized by a large and diverse community of microbes, referred as the gut microbiota. These microbes have the capacity to influence the body's physiology. The microbiota can also regulate different types of inflammatory processes during microbial infections. GF mice, having a sterile intestine, thus represent a suitable model for the study of the early stages of host-pathogen interactions at the naïve immunological background. We monitored the response of several cell subtypes during the first two days post infection at the site of infection (in peritoneum), and in one of the central lymphoid organs (in spleen). The parameter of cell subpopulations relative frequency demonstrated the basic differences between the response of GF and SPF mice to *F. tularensis* LVS infection. The Gr1^{+} and F4/80^{+} cells of GF mice in peritoneum demonstrated the mutual frequency changes in the two distinguishable waves. The data also demonstrate surprising behavior of lymphocytic cell subpopulations tested in the peritoneum and spleen. While the response of CD19^{+} cell subpopulations and CD3^{+} cell subpopulations of SPF mice changed their relative frequency at 48 h post infection, the same cell subpopulations of GF mice changed their relative frequency already at 12 h post infection. The comparison of the data collected from GF and SPF murine models demonstrate not negligible role of gut microbiota during the early stages of cellular response to intracellular bacterial pathogen.

This work was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense of Czech Republic and Specific research project no: SV/FVZ201707 from the Czech Ministry of Education, Youth and Sport.

P2-18
Seeking host targets of *Francisella* pathogenicity island effectors

Magdalena Proksova[1], Helena Rehulkova[1], Pavel Rehulka[1], Claire Lays[2], Jiri Stulik[1], Thomas Henry[2], Juraj Lenco[1]
[1]University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove, Czech Republic,[2]CIRI, International Center for Infectiology Research, Inserm U1111, CNRS, UMR5308, Lyon, France

Tularemia is caused by an intracellular, Gram-negative bacterium *Francisella tularensis.* Uniqueness of this bacterium is due to its ability to survive and replicate within the host phagocytic cells. Because of its extreme infectivity, ability to cause disease via inhalation route, and absence of a vaccine approved for human use, *Francisella tularensis* is classified as a potent biothreat agent. Proteins are involved in all biological processes in cells, we thus believe that interactions between host proteins and *Francisella* effector proteins is the key to full understanding of *Francisella* virulence. In our study, we seek to identify eukaryotic proteins targeted by putative *F. tularensis* effectors which were previously reported to be associated with type VI secretion system. Selected genes (VgrG, IglF and IglJ) were C- or N-terminally fused with FLAG tag and expressed in HEK293T cells. We employed affinity co-purification combined with stable isotope labeling with amino acids in cell culture (SILAC) to identify potential interaction partners of the effectors. In total, more than 900 eukaryotic proteins were identified using nano-scale liquid chromatography and high-resolution mass spectrometry. SILAC quantification has been helping us to filter out most of contaminating proteins from further considerations. Other experimental procedures will be utilized to corroborate our primary findings and the real role of confirmed protein-protein interactions in the *Francisella* virulence.

P2-19

Simultaneous Detection of *Francisella tularensis* and *Yersinia pestis* from Field Samples Using a Duplex Lateral Flow Recombinase Polymerase Amplification Assay (LF-RPA)

Aiping Qin[1], Yao Peng[2], Yanhua Wang[3], Jinxing Lu[2]
[1]National Institute For Communicable Disease Control And Prevention, Chinese Center For Disease Control And Prevention, Beijing, China, [2]National Institute for Communicable Disease Control and Prevention Chinese Center for Disease Control and Prevention, Beijing, China, [3]Department of Plague, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China

Tularemia is a plague-like disease caused by *Francisella tularensis* (*F.t.*), a gram negative intracellular bacterium known as one of the most pathogenic bacteria. This zoonotic pathogen invades more than 250 kinds of animals including human being. Infection of less than 10 colony formation unit (cfu) of *F.t.* type A SCHU S4 strain results in life-threatening illness to men if untreated. Occasional outbreaks of large number of dead feral animals in plague endemic foci were found *Y. pestis* (*Y.p.*) negative. Interestingly, when 12 marmot spleen samples were retrospectively analyzed by PCR and sequencing, 4 (33.3%) were *fop*A positive. We hypothesized that the etiologic agent of this kind of outbreaks could be caused by *F.tularensis*. Because it is a fastidious pathogen, isolation of *F.t.* from field sample usually ends with failure. A fast, sensitive and specific method to distinguish *Y.p.* from *F.t.* outbreak is urgently needed. Here we developed a duplex LF-RPA assay as an alternative to existing PCR-based methods for detection of *F.t.* and *Y.p.* at the same time. It doesn't need a complex device and the result can be visualized within 20 minutes. Sets of primers/probe specific to *F.t. tul*4 and *Y.p. pal* was designed with conjugation of biotin on 3' primers. Probes were labeled with either FAM (*F.t.*) or DIG (*Y.p.*). Specific primers/probe sets only detected *F.t.* or *Y.p.* respectively, but none of 10 selected other bacterial DNAs. The limit of detection(LOD) was about 500 cfu when the templates were prepared by boiling whole bacteria or 54fg with kit purified chromosomal DNA. Evaluation of this assay for field samples is in progress. The speed, simplicity, sensitivity and specificity of this duplex LF-RPA assay makes early accurate diagnosis of *F.t.* and *Y.p.* simultaneously at point of care possible.

P2-20
Persister formation in *Francisella tularensis*

Girija Ramakrishnan[1], Aiping Qin[2], Barbara Mann[1], Hiwot Abate[1]
[1]University of Virginia, Charlottesville, VA, [2]National Institute For Communicable Disease Control And Prevention, Chinese Center For Disease Control And Prevention, Beijing, China

Tularemia treatment with prevalent antibiotic therapy such as doxycycline may result in relapse. Relapses of bacterial infection are thought to originate with persister cells in the population that can tolerate exposure to lethal doses of antibiotics and re-establish infection once the antibiotic pressure is removed. We hypothesize that *Francisella tularensis* is able to integrate signals within the mammalian intracellular environment to form persisters. Using survival after exposure to a lethal dose of the antibiotic ciprofloxacin as readout, we determined that persisters are formed in actively growing cultures of both the Live Vaccine Strain (LVS) and the virulent strain Schu S4, and that the level of persistence is influenced by the growth medium. More persisters were formed in the stationary growth phase and under nutritional stress. We found that intracellular bacteria have a ten-fold or greater increase in persister formation relative to the infecting cultures. To better understand mechanisms that may regulate persistence within the host cell, we are evaluating persister formation in strains that are altered in their ability to escape the phagosome (Δ*mglA* and Δ*iglC*) and/or replicate within the macrophage (iron uptake deficient strains). Preliminary results suggest that signals perceived both within the phagosome and in the cytoplasm promote persister cell formation.

P2-21
Signaling in dendritic cells during *Francisella tularensis* invasion analyzed by phosphoproteomic approach

Pavel Rehulka[1], Ivo Fabrik[1], Marek Link[2], Daniela Fabrikova[1], Lenka Plzakova[1], Ivona Pavkova[1], Věra Vozandychová[1], Zuzana Krocova[1], Marina Šantić[3], Jiri Stulik[1]
[1]*University of Defence, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic,* [2]*FMHS UO, Hradec Kralove, Czech Republic,* [3]*Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Tularemia disease is caused by *Francisella tularensis*, a highly infectious Gram-negative bacterium. The delayed onset of the adaptive immunity followed by an excessive inflammation is characteristic for this infection disease. *Francisella* is capable of replication inside the primary phagocytes and avoiding their activation, which results in the impaired host response. The infected cells do not produce sufficient amount of pro-inflammatory stimuli and *Francisella* can use them as a niche for its multiplication. Dendritic cells (DCs) are important for antigen presentation and these migratory cells can also serve as *Francisella* hosts. To understand the response of the cells infected by *Francisella*, a quantitative phosphoproteomic approach for the analysis of intracellular signaling in DCs was used. The work was also focused on the beginning of the *Francisella* invasion, because these events play an important role in DCs activation and maturation. First, the primary bone marrow-derived DCs (BMDCs) were labeled using SILAC and then infected either by virulent *Francisella* strain (FSC200) or by an attenuated mutant lacking *dsbA* gene. Infected BMDCs were lysed in different time points and further analyzed using LC-MS after the enrichment for phosphorylated peptides. The bioinformatics analysis revealed that the majority of phosphosites differentially regulated during first 10 min after infection were related to the activation of PAKs, Akt-mTOR-p70S6K pathway and ERK signaling. No quantitative differences in BMDCs response were observed for both *Francisella* strains during the bacterial internalization. However, only the virulent *Francisella* strain induced a second phase of phosphorylation events in BMDCs 60 min after infection. This involved the activation of ERKs and p38 kinases and their downstream transcription factors. In conclusion, the results show that the second phase of phosphorylation may represent the initial event of DCs activation that differs between the virulent and attenuated *Francisella* strains.

Acknowledgement

The financial support of Czech Science Foundation (15-02584S) and a long-term organization development plan from the Faculty of Military Health Sciences, University of Defence, Czech Republic is gratefully acknowledged.

P2-22
Evaluation of antibiotic efficacy against planktonic and biofilm forms of *Francisella novicida*

Claire Siebert[1], Corinne Villers[2], **Patricia Renesto**[3]
[1]TIMC-IMAG UMR 5525 - UGA CNRS, CHU Grenoble Alpes, Grenoble, France, [2]Université de Caen Normandie, EA4655 U2RM, Caen, France, [3]TIMC-IMAG UMR5525, CNRS-UGA, Grenoble, France

Francisella tularensis is a Gram-negative bacteria responsible for tularemia, a re-emerging disease for which failures and relapses pose a serious threat in public health. The capacity of *Francisella* spp. to form biofilm was recently described and thought to be mainly a key mechanism for environmental survival and persistence. While environmental strains are indeed more prone to form biofilms than clinical isolates, we recently demonstrated that exposure of the *F. tularensis* LVS strain to ciprofloxacin generates resistant isolates with *fupA/B* mutations promoting the biofilm formation. This finding which could have crucial consequences, especially with respect to bacterial resistance and/or tolerance against antibiotics was never explored for bacteria from the *Francisella* genus. In this work, the time course of biofilm formation by *F. novicida* grown under static conditions was examined by confocal electronic microscopy, highlighting morphological changes of the bacteria embedded in the matrix. We then compared the efficacy of several antibiotics including ciprofloxacin, gentamycin and doxycycline on the survival of either planktonic or biofilm bacteria that was determined by both CFU quantification and by the measurement of the metabolic activity of the micro-organisms. The effect of dispersing compounds as EDTA was also investigated. Results obtained showed that antibiotic susceptibility of biofilm-growing bacteria are different from those of planktonic ones which could have significant diagnostic and therapeutic consequences.

P2-23

Galleria mellonella reveals niche differences between highly pathogenic and closely related strains of *Francisella* spp

Emelie Salomonsson[1], Johanna Thelaus[1], Eva Lundmark[1], Petter Lindgren[1], Andreas Sjödin[1], Mats Forsman[1]
[1]*FOI - Swedish Defence Research Agency, Umeå, Sweden*

Francisella tularensis, a highly virulent bacteria that causes the zoonotic disease tularemia, is considered a potential agent of biological warfare and bioterrorism. Although the host range for several species within the *Francisella* is known, little is known about the natural reservoirs of various *Francisella* species. The lack of knowledge regarding the environmental fates of these pathogens greatly reduces the possibilities for microbial risk assessments. The greater wax moth (*Galleria mellonella*) is an insect of the order *Lepidoptera* that has been used as an alternative model to study microbial infection during recent years. The aim of this study was to evaluate *G. mellonella* as a model system for studies of human pathogenic and closely related opportunistic and non-pathogenic strains within the *Francisella* genus. The employed *G. mellonella* larvae model demonstrated differences in lethality between human pathogenic and human non-pathogenic or opportunistic *Francisella* species. The *F. novicida*, *F. hispaniensis* and *F. philomiragia* strains were significantly more virulent in the *G. mellonella* model than the strains of human pathogens *F. t. holarctica* and *F. t. tularensis*. Our data show that *G. mellonella* is a possible in vivo model of insect immunity for studies of both opportunistic and virulent lineages of *Francisella* spp., that produces inverse results regarding lethality in *G. mellonella* and incapacitating disease in humans. The results provide insight into the potential host specificity of *F. tularensis* and closely related members of the same genus, thus increasing our present understanding of *Francisella spp*. ecology.

P2-24
The autophagy plays an important role in intracellular life of *Francisella* within *Dictyostelium* cells

Marina Šantić[1], Mateja Ozanic[1], Valerija Nora[1], Valentina Marecic[1], Mirna Mihelcic[1]
[1]*Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Autophagy or autophagocytosis is an evolutionally preserved catabolic process that involves the degradation of cytoplasmic components. During infection, various microorganisms regulate the autophagy process differently. Studies have shown that *F. novicida* in some cells stimulate autophagy, and in others suppress the autophagy process in favor of intracellular replication. Most of the studies were performed in mammalian cells, and very little is known about the autophagy process in the amoeba cell after infection with *Francisella*. It has been shown that in *Dictyostelium, F. noatunensis* subsp. *noatunensis* interacts with the autophagic machinery. There are no data about the role of autophagy on survival and replication of *F. novicida* and *F. tularensis* subsp. *holarctica* in *Dictyostelium discoideum.* In this study, we monitored the autophagy process in this social amoeba using different inducers and inhibitors of autophagy over infection with *F. novicida* and *F. tularensis* subsp. *holarctica.* The results have shown that treatment of cells with autophagy inhibitors, chloroquine and wortmannin has a negative effect on survival, replication and intracellular trafficking of both strains of *Francisella.* In contrast, induction of autophagy in amoebae cells results of high number of intracellular bacteria and successful replication in *Francisella* containing vacuole. We can conclude that the formation of an autophagic vacuole support the intracellular lifestyle of *Francisella* within *Dictyostelium discoideum*.

P2-25
Unique very long chain acyl containing *Francisella tularensis* phospholipids suppress inflammation

Benji Schwarz[1], Robin Ireland[1], Forrest Jessop[1], Glenn Nardone[2], Catharine Bosio[1]
[1]Rocky Mountain Laboratories, NIAID/NIH, Hamilton, MT, [2]National Institutes of Health, Bethesda, MD

Intracellular bacterial pathogens must interface intimately with host systems to prevent detrimental immune responses. *Francisella tularensis* (FT) is a gram-negative intracellular pathogen with the ability to cause rapid, lethal disease in humans. FT and FT-derived molecules actively impair inflammatory responses as a mechanism of virulence. In particular, lipid fractions from FT have been previously shown to be potently immunosuppressive. Here we demonstrate that unique acyl motifs in the phospholipids from FT are responsible for this lipid-mediated immune suppression. We have confirmed that the FT lipid extract contains phosphotidylethanolamine (PE), phosphotidylcholine (PC), cardiolipin (CL) and phosphatidylglycerol (PG) species containing very long chain, saturated, acyl tails ($\geq C_{22}$). Administration of isolated fractions of PE, PC and CL from FT to macrophages in culture confirmed that all of these species are immune suppressive though PE accounts for the vast majority of phospholipids in the organism. Administration of synthetic versions of very long chain acyl PE or PC species recapitulated the immune suppression observed in the raw lipid extract or the purified phospholipid fractions. Many diseases are complicated or exacerbated by excessive inflammation. As such novel anti-inflammatory compounds are of significant interest toward developing therapeutics. Ft lipid or a synthetic liposome consisting of very long chain acyl containing PE and PC were administered to human dendritic cells challenged with Dengue virus. Inflammatory cytokine production by Dengue infected cells was reduced in cells treated with lipid. These results suggest that this very long chain acyl phospholipid motif has utility in the development of novel therapeutics as well as further understanding the immune silent character of early Ft infections.

P2-26
Protein production in *Francisella tularensis LVS* under control of TetR inducible system

Valeria Sesko (Sheshko)[1], Igor Golovliov[2], Jiri Stulik[3]
[1]Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic, [2]Umeå University, UMEÅ, Sweden, [3]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

Francisella tularensis is a gram-negative intracellular pathogen causing tularemia in humans and a large number of animal species. Because of the low infectious dose, high virulence and environmental resistance, it is considered to be one of the most infectious bacteria. These properties are also a reason for fear of misusing the microbe as a potential biological weapon. The development of a safe and effective vaccine for military and medical personnel as well as for civilian population is very important. We focused on the identification of bacterial proteins with significant immunogenic potential that could be used to design the subunit vaccine against the *F.tularensis* infection. The development of the protein production system in *F. tularensis*, which will maintain all posttranslational modifications, is another necessary step in the preparation of an effective subunit vaccine against *F. tularensis*. Fully functional proteins can be used for immunization, host-pathogen interaction studies, modifications and structure determination, as well as definition their biological function. Since the tetracycline-inducible system has been used in *F.tularensis* to effectively regulate gene expression under defined conditions we decided to test the functionality of this systém for controlled protein expression in *F.tularensis LVS*. We used this inducible system where a tet operator sequence was cloned into modified *F.tularensis* groESL promoter sequence and carried in plasmid that constitutively expressed TetR. Based on previous results of proteomic and immunological studies, immunogenic proteins with different cell localization, structure and molecular properties were selected and cloned under the hybrid *Francisella* tetracycline-regulated promoter (*FTRp*). Transcription was initiated with addition of anhydrotetracycline (ATc). Expression level of proteins, producing in *F.tularensis* LVS, was analysed by Western blot. As a result, we were able to produce and subsequently purify selected proteins of *F.tularensis*.

P2-27

Francisella tularensis D-Ala D-Ala carboxypeptidase DacD is involved in intracellular replication and it is necessary for bacterial cell wall integrity

Petra Spidlova[1], Pavla Stojkova[1], Vera Dankova[2], Iva Senitkova[1], Marina Šantić[3], Dominik Pinkas[4], Vlada Philimonenko[5], Jiri Stulik[1]
[1]Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic, [2]FMHS UO, Hradec Kralove, Czech Republic, [3]Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [4]Institute of Molecular Genetics ASCR v.v.i., Microscopy Center, Electron Microscopy Core Facility, Prague, Czech Republic, [5]Institute of Molecular Genetics ASCR v.v.i., Department of Biology of the Cell Nucleus, Prague, Czech Republic

D-alanyl-D-alanine carboxypeptidase, product of *dacD* gene in *Francisella*, belongs to penicillin binding proteins (PBPs) and is involved in remodeling of newly synthetized peptidoglycan. In *E. coli*, PBPs are synthetized in various growth phases and they are able to substitute each other to a certain extent. The DacD protein was found to be accumulated in fraction enriched in membrane proteins from severely attenuated *dsbA* deletion mutant strain. It has been presumed that the DsbA is not a virulence factor by itself but that its substrates, whose correct folding and topology are dependent on the DsbA oxidoreductase and/or isomerase activities, are real virulence factors. Here we demonstrate that *Francisella* DacD is required for intracellular replication and virulence in mice. The *dacD* insertion mutant strain showed higher sensitivity to acidic pH, high temperature and high osmolarity when compared to the wild-type. Eventually, transmission electron microscopy revealed differences in both the size of mutant bacteria and also defects in outer membrane underlying its SDS and serum sensitivity. Taken together these results suggest DacD plays an important role in *Francisella* pathogenicity.

P2-28
Macrophages acquire cytosolic material from live, infected cells as part of an antimicrobial response

Shaun Steele[1], Amanda Foreman[1], Thomas Kawula[1]
[1]*Washington State University, Pullman, WA*

In the course of studying the cell to cell transfer of *F. tularensis,* we found that macrophages and dendritic cells can acquire small pieces of neighboring cells without killing the donor cell. The acquired material contains portions of the donor cell's cytosol, which can include lipid droplet, functional mitochondria, antigens and even intact intracellular bacteria. During infections with *F. tularensis,* the bacteria acquired by the recipient macrophage are able to replicate within the newly infected cell. An open question is why macrophages acquire infectious material from neighboring cells if bacterial acquisition results in enhanced bacterial replication and dissemination. We found that macrophages undergo increased cytosolic acquisition in response to a wide range of bacterial species. Further, stimulation of Toll-like receptor 4 or C-type lectin receptors increase the amount of cytosolic material acquired by a recipient macrophage. The enhanced cell-cell transfer during infection correlated with an increase in the cell-cell binding integrins CD44, ICAM-1, and both components of the heterodimer VLA-4. Immediately following transfer, newly acquired cytosolic material and bacteria were enclosed in an endocytic structure. Endosomes containing transeferred cytosolic material were acidified, indicating the material was degraded. This observation suggests that the recipient macrophage can contribute to antigen presentation as well as enhancement of an innate and adaptive immune response to infection. However, in the case of *Francisella* infection, we found that, *F. tularensis* escapes the endosome in the recipient cell with similar kinetics as typical phagosomal escape. Thus, *F. tularensis* can successfully exploit cytosolic acquisition by macrophages to expand their replicative niche.

P2-29
HU protein is involved in intracellular growth and full virulence of *Francisella tularensis*

Pavla Stojkova[1], Petra Spidlova[1], Juraj Lenco[1], Helena Rehulkova[1], Lucie Kratka[1], Kamil Hercik[1], Jiri Stulik[1]
[1]University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove, Czech Republic

The nucleoid-associated HU proteins are small abundant DNA-binding proteins in bacterial cell which play an important role in the initiation of DNA replication, cell division, SOS response, control of gene expression and recombination. HU proteins bind to double stranded DNA non-specifically, but they exhibit high affinity to abnormal DNA structures as four-way junctions, gaps or nicks, which are generated during DNA damage. In many pathogens HU proteins regulate expression of genes involved in metabolism and virulence. Here, we show that the *Francisella tularensis* subsp. *holarctica* gene locus FTS_0886 codes for functional HU protein which is essential for full *Francisella* virulence and its resistance to oxidative stress. Further, our results demonstrate that the recombinant FtHU protein binds to double stranded DNA and protects it against free hydroxyl radicals generated via Fenton´s reaction. Eventually, using an iTRAQ approach we identified proteins levels of which are affected by the deletion of *hupB*, among them for example *Francisella* pathogenicity island (FPI) proteins. The pleiotropic role of HU protein classifies it as a potential target for the development of therapeutics against tularemia.

P2-30
Undetected ongoing tularemia outbreak identified as new endemic foci in the Western Georgia, 2016-2017

Maka Tsilosani[1], Khatuna Zakhashvili[1], Marika Geleishvili[2], Naile Malakmadze[3], Gocha Giorgidze[4], Giorgi Chakhunashvili[1], Nikoloz Tsertsvadze[1], Mariam Izoria[1], Maia Maxaradze[1], Paata Imnadze[1]

[1]National Center For Disease Control and Public Health, Tbilisi, Georgia, [2]Centers for Disease Control and Prevention – South Caucasus office, Tbilisi, Georgia, [3]Northrop Grumman, Atlanta, USA, Atlanta, Georgia, [4]National Center For Disease Control and Public Health, Kutaisi, Georgia

Background: Although, Eastern Georgia was considered as the endemic foci of tularemia, there was no evidence of the disease in Western Georgia until 2017. In March of 2017, three patients residing in village Chkhari, Terjola region of Western Georgia were diagnosed with tularemia. The goal of investigation was to identify additional cases, magnitude and source of the outbreak to prevent spread of the disease. ***Methods:*** Retrospective investigation was performed in the affected part of the village. Standard questionnaire was used to interview respondents and blood samples were tested serologically for *F. tularensis* (ELISA and MAT). Bacteriological and PCR research was conducted in one patient. A case was defined as individual residing in Chkhari from September 2016 to April 2017, who had one or more symptoms of any form of tularemia and an increase of antibodies in serum. Environmental samples *(Ixodes* ticks, hay and oat seeds) were tested by bacteriological, serological and PCR methods for *F. tularensis*.

Results: Out of 49 interviewed persons 9 had symptoms of tularemia including 5 - oropharyngeal, 2 - ulceroglandular, one oculoglandular and one gastrointestinal manifestation. All cases were between 20 to 59 of age. Earliest date of symptom onset was on 15th of September, 2016 and latest -on 15th of April, 2017 with a peak in February (4 cases) when first time tularemia was suspected. Blood samples obtained from 49 persons were tested by ELISA and MAT. 17 patients tested ELISA-IgM positive (including 8 cases with no symptoms), 2 - by MAT and one - by PCR. Environmental samples were negative for *F. tularensis.* Epidemiological links among cases were not revealed. All 9 persons reported one or more exposure to hay and oat seeds transported form Eastern Georgia, hunted animals or insects.

Conclusion: Undetected tularemia outbreak that lasted for 8 months in the area with no previous cases was reported in Western Georgia. A possible source might be an exposure to hay and oats seeds obtained from endemic zones. No further cases were revealed after informing local population on tularemia prevention and conducting rodent control measures. To increase awareness of physicians and include tularemia in differential diagnosis was recommended.

P2-31
Lysine acetylation modulates chitinase activity in *Francisella novicida*

Monique van Hoek[1], Ekaterina Marakasova[2], Alexandra Ii[2], Kristina Nelson[3]
[1]George Mason University, Centreville, VA, [2]George Mason University, Manassas, VA, [3]Virginia Commonwealth University, Richmond, VA

Post-translational modifications (PTMs) generally refer to covalent addition of a chemical group to a protein by an enzyme. There are more than 200 known PTMs that result in protein mass change, hydrophobicity, and charge that influence protein folding and conformation. PTMs have been reported for a very small number of *Francisella tularensis* proteins to date. In order to fill this gap of knowledge, we performed a proteome-wide analysis of lysine acetylation in *Francisella novicida* strain U112. Following *in vitro* acetylation and trypsin digestion of *Francisella* proteins, we performed LC/MS/MS to identify acetylation PTMs. This "bottom-up" proteomics approach allowed us to discover acetylation of many *F. novicida* proteins, including two chitinases and chitin-binding protein. We have previously shown that Chitinase A and Chitinase B are involved in biofilm formation in *F. novicida* through modulation of the bacterial surface properties. In vitro lysine acetylation results in the down-regulation of chitinase activity. Consequently, lysine acetylation of chitinases and chitin binding protein may represent a novel mechanism of regulation of biofilm formation in *F. novicida* and allow cells to rapidly respond to changing environmental conditions.

P2-33
Development of an ELISA to measure plasma antibody responses to tularemia vaccines

Katherine Willett - O'Malley[1], Jennifer Bowling[1], Elizabeth Stinson[1], Amy Caroline[1], Karsten Hazlett[2], Eileen Barry[3], Doug Reed[1]
[1]University of Pittsburgh, Pittsburgh, PA, [2]Albany Medical College, Albany, [3]University of Maryland at Baltimore, Baltimore, MD

There are currently no licensed tularemia vaccines, although a number of efforts are underway to develop one. Understanding how tularemia vaccines protect and developing immune correlates that can predict protection in animals and humans is critical to this effort. To evaluate tularemia vaccines in New Zealand White rabbits, we developed an indirect chemiluminescence-based ELISA that measures plasma IgG, IgM, and IgA responses against Francisella tularensis using whole, heat-killed SCHU S4 as the target antigen. Hyperimmune plasma from a surviving rabbit was used as a positive control. Plasma antibody titer was determined by four-parameter logistical regression to determine the median effective concentration (EC50) and slope. Antibody titers rise post-vaccination, peaking on day 21 post-primary vaccination. Comparing vaccinated rabbits across a number of vaccines and vaccine regimens, plasma IgG and IgM EC50 titers before challenge corresponded with survival of vaccinated rabbits after challenge with aerosolized SCHU S4. Plasma IgA EC50 titer, in contrast, did not correspond with protection of vaccinated rabbits. We are currently further developing and characterizing this assay for potential use in clinical trials. Using rabbits with a range of antibody responses to vaccination, we evaluated the effect of varying sample and secondary antibody incubation time and temperature. Temperature did not significantly affect EC50 values for individual rabbits but did affect the slope and maximum light units. We are also evaluating other methods for quantifying antibody titer including the WHO tularemia standard (reading at 1:1000) and traditional ELISA endpoint titer. These results and additional efforts at optimizing and validating this assay for use in clinical trials will be reported and discussed.

P2-34
Use of Zebrafish as Vaccine and Infection Model for *Francisella*

Hanne Winther-Larsen[1], Anders Sjosted[2], Espen Brudal[3], Lilia Ulanova[1], Elisabeth Lampe[1], Julia Tandberg[1], Leidy Lagos[4], Gareth Griffiths[1]
[1]University of Oslo, Oslo, Norway, [2]Department of Clinical Microbiology, Bacteriology, and Laboratory for Molecular Infection Medicine Sweden, Umea, Sweden, [3]Zoetiz, Oslo, Norway, [4]Norwegian University of Life Science, Oslo, Norway

Infections by the Gram-negative intracellular bacterial pathogens, *Francisella*-cause disease in a range organisms. *Francisella tularensis* subspecies cause disease in mammals including humans, while *F. noatunensis* subsp is one of the main factors hampering the development of fish farming based on Atlantic cod in Norway and is deleterious to tilapia, a farmed fish that is produced over 3.5 mill tons/year. In fish the immunologically and experimentally inaccessible intracellular location of *Francisella* have until now complicated the development of protective measures. A limiting factor in the development of any vaccine against aquaculture-relevant fish species is the technical difficulty in working with these fish under laboratory conditions, especially the relatively long developmental time that hampers screening of different vaccine formulations. To overcome this problem we have established both embryo and adult zebrafish as a cheap, rapidly developing model organism for the analysis of fish infections. In addition, a zebrafish embryo infection model has also been developed for *F. novicida*, a commonly used model for *Francisella* infection in mammals. A particular powerful aspect of this fish is its optical transparency, allowing fluorescently labeled bacteria and the fish immune cells to be visualized non-invasively in real time. We have successfully used this model to develop and screen the effect of vaccines developed in our laboratory against infection caused by *F. noatunensis.* Based on these results the most promising vaccine candidates will further be tested in the natural fish hosts of the infection.

P2-35
Epidemiology and Ecology of Tularemia in Switzerland in a high resolution phylogenetic context.

Matthias Wittwer[1], Ekkehardt Altpeter[2], Paola Pilo[3], Sebastian Gygli[4], Urs Karrer[5], Christian Beuret[1], Rahel Ackermann[1], Daniela Jacob[6], Roland Grunow[6], Nadia Schuerch[1]

[1]Spiez Laboratory, Spiez, Switzerland, [2]Federal Office of Public Health FOPH, Bern, Switzerland, [3]Institute of Veterinary Bacteriology, University of Berne, Bern, Switzerland, [4]Swiss Tropical and Public Health Institute, Basel, Switzerland, [5]Cantonal Hospital Winterthur, Winterthur, Switzerland, [6]Robert Koch Institute, Berlin, Germany

As seen in other European countries, tularemia is on the rise in Switzerland drawing attention of public authorities and media to the disease. In this scope, the presented work provides insights into the population structure and epidemiology of *Francisella tularensis* subsp. *holarctica* (*Fth*), the causative agent of tularemia in Switzerland. *Fth* isolates were obtained from castor bean ticks (*Ixodes ricinus)*, animals and humans throughout Switzerland and high resolution phylogeny was inferred using whole genome sequencing (WGS). The application of WGS allows to describe the complex transmission patterns of the disease and to map the genetic diversity of the monophyletic *F.t. holarctica* population on a micro-geographic scale. The majority of the *F. tularensis* subsp. *holarctica* population in Switzerland belongs to the west European B.11 clade and shows an extraordinary genetic diversity underlining the old evolutionary history of the pathogen in the alpine region.The combined analysis of the epidemiological data of human tularemia cases with the whole genome sequences of *Fth* isolates provide evidence that ticks play a pivotal role in transmitting *F. tularensis* subsp. *holarctica* to humans and other vertebrates in Switzerland. This is further underlined by the correlation of disease risk estimates with climatic and ecological factors influencing the survival and vector competence of ticks.

P2-36

A phosphatidylinositol 3-kinase effector alters phagosomal trafficking to promote intracellular growth of *Francisella*

Hannah Ledvina[1], Katherine Kelly[1], Aria Eshraghi[1], Rachael Plemel[2], Snow Peterson[1], Brian Lee[3], Shaun Steele[4], Marlen Adler[1], Thomas Kawula[4], Alexey Merz[2], Shawn Skerrett[3], Jean Celli[4], Joseph Mougous[1]

[1]Department of Microbiology, University of Washington School of Medicine, Seattle, Washington, [2]Department of Biochemistry, University of Washington School of Medicine, Seattle, [1]Department of Microbiology, University of Washington School of Medicine, Seattle, [3]Division of Pulmonary, Critical Care and Sleep Medicine, Harborview Medical Center, Seattle, [4]Paul G. Allen School for Global Animal Health, Washington State University, Pullman WA

The success of intracellular bacteria relies on their ability to establish and maintain a permissive niche. By mechanisms that have remained undefined, a *Francisella* pathogenicity island-encoded secretion system allows bacteria in this genus to escape from phagosomes and replicate within the host cell cytoplasm. At this conference, I will present our discovery that a substrate of this secretion system, OpiA, represents a previously undescribed family of wortmannin-resistant bacterial phosphatidylinositide (PI) 3-kinase enzymes. Representatives of this family can be found in a wide range of intracellular pathogens, including *Rickettsia* and *Legionella* spp, as well as in many environmental bacteria. I will show that OpiA is recruited to endocytic membranes by binding PI(3)-phosphate (PI(3)P) and that it acts on the *Francisella*-containing phagosome to promote bacterial escape. Finally, I will present experiments demonstrating that arrest of endosomal maturation mitigates the phenotypic consequences of OpiA inactivation. In total, our findings suggest that Francisella, and likely other intracellular bacteria, override the finely-tuned dynamics of endosomal PI(3)P in order to delay phagosome maturation and promote intracellular survival and pathogenesis.

P2-37
Identifying virulence factors secreted by *Francisella tularensis* during infection

Peter Benziger[1], Erik Kopping[1], David Thanassi[1]
[1]*Stony Brook University, Stony Brook, New York*

Francisella tularensis is a successful pathogen in part because of its ability to invade a variety of host cells and replicate intracellularly following phagocytosis. While several virulence factors have been identified as critical for *Francisella* pathogenesis, how the bacteria manipulate the host cell to maintain their intracellular niche remains to be fully elucidated. To date, genes encoding both a type I secretion system (T1SS) and type VI secretion system (T6SS) have been identified in *F. tularensis* as potential mechanisms for delivering virulence factors to host cells. Recent evidence indicates that phagosomal escape is dependent on the T6SS; however, the repertoire of substrates secreted via the T6SS during infection is unclear. Additionally, our lab has identified TolC in *F. tularensis* as important for the bacteria's ability to delay host cell death. TolC is an outer membrane channel protein of the T1SS found across various Gram-negative bacteria and is associated with virulence. We found that the loss of TolC during infection leads to increased cytotoxicity and premature host cell death compared to the wild-type bacteria. In addition, the absence of TolC during an *in vivo* mouse infection model leads to decreased virulence. Therefore, we hypothesize that TolC functions in virulence protein secretion during the cytosolic stage of growth to delay host cell death. To address our hypothesis, we have employed bio-orthogonal non-canonical amino acid tagging (BONCAT) in combination with click chemistry to detect proteins secreted during infection through TolC. In brief, we have engineered *F. tularensis* with a tRNA synthase to incorporate chemically modified methionine during protein synthesis. A biotin-alkyne tag can then be covalently bound to the modified methionine, allowing for bacterial proteins secreted into the host cytosol to be detected by streptavidin. Identifying effectors secreted by *Francisella* will be critical in furthering our understanding of *F. tularensis* pathogenesis and revealing mechanisms by which it manipulates host cells responses.

P2-38
The role of the dynamic Type VI Secretion System in *Francisella* virulence

Maj Brodmann[1], Roland Dreier[1], Petr Broz[2], Marek Basler[1]
[1]*Biozentrum, University of Basel, Basel, Switzerland,* [2]*University of Lausanne, Lausanne, Switzerland*

The model organism *Francisella tularensis* subspecies *novicida* (*F. novicida*) is frequently used to study the fatal zoonotic disease tularemia and *Francisella* virulence. The advantage of *F. novicida* is that it harbors only one *Francisella* Pathogenicity Island (FPI). The FPI is critical for phagosomal escape and thus *Francisella* virulence and encodes a non-canonical Type VI Secretion System (T6SS). Hallmark of the T6SS is the dynamic assembly of a long cytosolic sheath and its rapid contraction to deliver effector proteins across target cell membranes of both bacterial and eukaryotic cells. Intriguingly, the FPI lacks several conserved components including the ATPase ClpV, which is essential for disassembly of contracted sheaths and T6SS dynamics in canonical T6SS. On the other hand, the FPI encodes many *Francisella* specific genes with unknown function. This unique gene composition raised our interest in understanding the assembly and dynamics of *Francisella* T6SS. Here we used live-cell fluorescence microscopy to show that in *F. novicida* the T6SS dynamics of assembly and disassembly on agar pads and in live macrophages are similar to dynamics of canonical T6SS. Strikingly, *Francisella* T6SS is preferentially located at the bacterial poles. General-purpose unfoldase ClpB co-localizes with the contracted T6SS sheath and is required for its disassembly. We analyzed T6SS dynamics in in-frame deletion mutants lacking genes with unknown function. Thereby, we identified components necessary for assembly (*iglF*, *iglG*, *iglI* and *iglJ*) and two potential effectors (*pdpC* and *pdpD*), which are not required for T6SS dynamics but bacteria lacking these two genes are unable to escape the phagosome, activate AIM2 inflammasome or cause disease in mice.

Expression plasmids are difficult to use in *F. novicida*, as they are prone to spontaneous deletions or impeded by restriction-modification systems. To overcome this, we constructed two mobilizable and tunable expression plasmids pJB1 and pJB2 with different ranges of maximum expression levels. Both plasmids contain a tetracycline inducible promoter to control expression levels and *oriT* for RP4 mediated mobilization. pJB1 and pJB2 were used to restore T6SS activity in *iglF*, *iglI* and *iglC* deletion mutants, excluding the possibility of polar effects on downstream genes. High efficiency of transfer and tunable expression of these plasmids will facilitate future studies in *F. novicida*.

P2-39

ClpB mutants of *Francisella tularensis* subspecies *holarctica* and *tularensis* are defective for type VI secretion and intracellular replication

Athar Alam[1], Igor Golovliov[2], Eram Javed[1], **Anders Sjöstedt**
[1]Department of Clinical Microbiology, Umeå, Sweden, [2]Umeå University, UMEÅ, Sweden

Recent data suggest that the HSP100 family member, ClpB, is involved in T6SS disassembly in the subspecies *Francisella novicida*. Here, we investigated the role of ClpB for the function of the T6SS and for phenotypic characteristics of the human pathogenic subspecies *holarctica* and *tularensis*. The *ΔclpB* mutants of the human live vaccine strain, LVS, and the highly virulent subspecies *tularensis* SCHU S4 strain, both showed extreme susceptibility to heat shock and low pH, severely impaired type VI secretion (T6S), and significant, but impaired intracellular replication compared to the wild-type strains. Moreover, they showed essentially intact phagosomal escape. Infection of mice demonstrated that both *ΔclpB* mutants were highly attenuated, but the SCHU S4 mutant showed more effective replication than did the LVS strain. Collectively, our data demonstrate that ClpB performs multiple functions in the *F. tularensis* subspecies *holarctica* and *tularensis* and its function is important for T6S, intracellular replication, and virulence.

P2-40
Fragmentary analysis of chitinase gene cluster and its expression in *Francisella tularensis* strains of different subspecies

Elena Kulikalova, Ayuna Syngeeva, Valerii Nikolaev, Eduard Goldapel, Andrey Mazepa, Aleksandra Korneva, Evgenii Markov, Sergey Balakhonov
[1]*Irkutsk Antiplague Research Institute of Siberia and Far East, Irkutsk, Russian Federation*

Background: *Francisella tularensis* circulating in multicomponent biocenoses is the cause of epidemic outbreaks and epizooties. One of mechanisms of *F. tularensis* preservation in environment is its interaction with chitin-containing surfaces by means of chitinase enzymes. **Objective.** Molecular-genetic detection of *F. tularensis* chitinase gene cluster, bioinformational analysis of its nucleotide sequence and screening of chitinolytic activity.

Materials/methods: *F. tularensis* subsp. *holarctica* I-250, I-391, 15V, I-126; subsp. *mediasiatica* 385, B-7892; subsp. *novicida* 383, 384 strains were used. Fragments of gene with *chiA*, *chiB*, *chiC*, *chiD* primers were amplified. Amplicons were detected with the subsequent purification using the Exonuclease I set (Thermo scientific, USA). Sequencing was performed using BrightDyeTerminator Cycle Sequencing kit (Nimagen, Netherlands) on Genetic Analyzer device, Applied Biosystems. Sequences were compared with the sequences from GenBank database. Chitinolytic activity of the strains was assayed on suspensions of the intact cells and their lysates in a gel diffusion assay based on the size of the hydrolysis zone formed. The samples in concentration of 20×10^9 cells ml^{-1} were pipeted with three replications into wells of agarose gel plate with colloid chitin as a substratum.

Results: Sequences of *chiA*, *chiB*, *chiC*, *chiD* genes from all studied *F. tularensis* strains were sequenced. Comparison with the GenBank data showed 100 and 99 % homology of the genes within species and subspecies respectively. Nucleotide sequences of *chiA* gene from *F. tularensis* 15B strain in size 601 bp were deposited to GenBank numbered KY563318.1, I-250 (*chiA* MG728099.1); *F. tularensis* subsp. *mediasiatica* B-7892 (chiA MF984201, chiD MF363049.1, *chiA* MF984202, *chiC* MG728102.1, *chiC* MF984202.1, *chiA* MF984201.1); 385 (*chiC* MF398390.1). All samples of the cell suspensions and its lysates demonstrated various degree of chitinase activity. Suspensions and lysates of *F. novicida* strains revealed the most expressed chitinolytic activity.

Conclusions: The chitinase genes of four species were defined by sequencing in the strains taken in the experiment. Screening of the strains revealed the ability to produce chitinases in all subspecies of tularemia microbe. Identified chitinolytic enzymes participate in the adhesion of *F. tularensis* to chitin-containing environmental objects and provide access of nutrient substratum to the cells under unfavorable environment conditions.

Session 5

Human Infection and Treatment

S5-1
An uncommon outbreak of tularemia after a wine grape harvest in fall 2016, Germany, and molecular identification of the outbreak 'strain' as well as of the source of infection

Roland Grunow[1], Daniela Jacob[1], Kristin Koeppen[1], Aleksander Radonić[1], Berit Haldemann[1], Philipp Zanger[2], Timo Wolf[3], Klaus Heuner[1]
[1]Robert Koch Institute, Berlin, Germany, [2]Institute for Infectious Disease Prevention Landau, Federal State Agency for Consumer & Health Protection Rhineland-Palatinate, Landau, Germany, [3]University Hospital Frankfurt, Frankfurt, Germany

End of 2016, an uncommon outbreak of tularemia occurred in humans in Germanyt after a grape harvest. Six (2 females, 4 males; median age 24.5 years, interquartile range 10.3-39.5) out of 29 grape harvesters showed first symptoms (swollen cervical lymph nodes, fever, chills, difficulties swallowing, diarrhea) four to eight days after the suspected exposure and subsequently developed antibodies against the lipopolysaccharide of *F. tularensis* (detected by ELISA and Western-Blot). A multivariable analysis revealed that drinking fresh must remained the only significant predictor for acquiring tularemia (aIRR=13.5 p=0.014). Because tularemia was initially not considered, a delay of about five weeks occurred in confirming the diagnosis of tularemia. *F. tularensis* DNA could be detected using a specific real-time PCR from pus of the lymph node of 46 year old patient with a protracted course of infection. By quantitative PCR, genome equivalents were determined: In winery W1 (place of outbreak), wine sort-A (16,849 GE/ml) and sort-B (440 genome equivalents/ml) was pressed after suggesting cross-contamination on the press. Wine sort-C (1 GE/ml) from winery W2 was collected by the same mechanized harvester later that day suggesting cross-contamination via the harvester. Quantitative-PCR results revealed a substantial contamination of sort-A by 10^9–10^{10} *F. tularensis* in 730 liters. The pathogen was not detected in an unrelated sample sort-D.

A bacterial isolate could not be obtained. Using extracted DNA, *Francisella tularensis* subsp. *holarctica* belonging to the phylogenetic clade B.12/B.34 (Erythromycin-resistant Biovar II) could be identified. By direct next generation sequencing, nearly the whole genome sequence of the causative *Francisella* strain could be identified. The patient had been most likely infected with the same genotype of *Francisella* present in the wine product. Furthermore, a vertebrate-specific cytochrome b PCR identified mitochondrial DNA of *Apodemus sylvaticus* (wood mouse) in the wine products, suggesting that a wood mouse infected with *F. tularensis* may have been responsible for contaminating the must.

In conclusion, the discovered source of infection and the transmission scenario of *F. tularensis* in this outbreak have been observed for the first time and suggest the need for additional hygienic precaution measures when consuming freshly pressed must.

S5-2
Transmission of *Francisella tularensis* by Solid Organ Transplantation

Christina Nelson[1], Christian Murua[2], Jefferson Jones[3], Kelli Mohler[4], Ying Zhang[5], Landon Wiggins[4], Natalie Kwit[6], Laurel Respicio-Kingry[7], Luke Kingry[8], Jeannine Petersen[9], Jennifer Brown[10], Saima Aslam[11], Melissa Krafft[11], Shadaba Asad[12], Hikmat Dagher[13], John Ham[12], Luis Medina-Garcia[12], Kevin Burns[14], Walter Kelley[15], Alison Hinckley[7], Pallavi Annambhotla, Karen Carifo[5], Anthony Gonzalez, Elizabeth Helsel[4], Curtis Fritz, Sridhar Basavaraju
[1]CDC, Fort Collins, CO, [2]Southern Nevada Health District, Las Vegas, Las Vegas, [3]Centers for Disease Control and Prevention, Atlanta, [4]Phoenix Area Indian Health Service, Phoenix, [5]Southern Nevada Health District, Las Vegas, [6]Vermont Department of Health, Burlington, [7]CDC, Fort Collins, [8]Centers for Disease Control and Prevention, Fort Collins, [9]Centers for Disease Control and Prevention, Fort Collins, CO, [10]University of California Davis Medical Center, Davis, [11]University of California, San Diego, San Diego, [12]University Medical Center of Southern Nevada, Las Vegas, [13]Sunrise Hospital and Medical Center, Las Vegas, [14]Nevada Donor Network, Las Vegas, [15]American Red Cross, Salt Lake City CDC, Atlanta Sacramento County, Sacramento, CA, California Department of Public Health, Sacramento

Background In July 2017, one heart recipient and two kidney recipients from a common donor developed fever and sepsis; one of the kidney recipients died. Tularemia was suspected only after blood cultures from the surviving kidney recipient grew a *Francisella* species. We conducted an investigation to determine the transmission source and identify other at-risk patients.

Methods

We reviewed medical records, conducted interviews with the donor's next of kin, and performed an environmental investigation of the donor residence and surrounding area. Blood and tissue samples from the donor and recipients and environmental samples were tested for *F. tularensis*.

Results

The organ donor, a male in his forties from the southwestern United States, was hospitalized for acute alcohol withdrawal syndrome, pneumonia, and sepsis. Despite treatment with broad-spectrum antibiotics, he developed multi-organ system failure and was declared brain-dead. Following his death, the heart and both kidneys were recovered and transplanted into three recipients, all of whom became ill. One of the kidney recipients died 5 days post-transplant. *F. tularensis* subspecies *tularensis* (clade A2) was cultured from the blood of archived donor spleen tissue and both kidney recipients. Whole genome multi-locus sequence typing demonstrated the isolated strains were indistinguishable. The heart recipient

remained seronegative with negative blood cultures but had been receiving antibiotics prior to transplant for a medical device infection. Two lagomorph carcasses found near the donor's residence also tested positive by PCR for *F. tularensis* subspecies *tularensis* (clade A2).

Conclusions

This is the first known transmission of *F. tularensis* via solid organ transplantation. Detection of tularemia in patients can be challenging due to nonspecific clinical manifestations, widespread but sporadic nature of human cases, and limited sensitivity of blood culture. It is important for clinicians to be aware of possible *F. tularensis* infection in organ donors and recipients and report suspected cases to public health authorities for investigation.

S5-3
Tularemia in France: a ten-year overview and unusual clinical aspects

Yvan Caspar[1], Isabelle Pelloux[2], Sandrine Boisset[3], Patricia Renesto[4], Max Maurin[5]
[1]Grenoble Alpes University Hospital, Grenoble, France, [2]Centre Hospitalier Universitaire Grenoble-Alpes, Centre National de Référence des Francisella, CS 10217, 38043 Grenoble Cedex 9, France, Grenoble, France, [3]Université Grenobles Alpes, Grenoble, France, [4]TIMC-IMAG UMR5525, CNRS-UGA, Grenoble, France, [5]CHU Grenoble Alpes, Université Grenoble Alpes, Grenoble, France

As the French reference center for human tularemia, we investigated 1960 suspected cases of tularemia between 2007 and 2017. We confirmed tularemia diagnosis in 253 cases, corresponding to 175 male and 78 female patients (sex ratio, 2.24; mean age 49.5years). Among them, 165 were confirmed cases (58 by culture, 103 by PCR, and 15 by seroconversion or fourfold increase in antibody titers between acute ad convalescent phase sera). The remaining 88 cases were probable (a single positive serological titer in a patient with compatible clinical and epidemiological data). All culture- or PCR-confirmed cases were demonstrated to be infected with *F. tularensis* subsp. *holartica* biovar I. The clinical presentations mainly corresponded to classical forms of tularemia: ulceroglandular or glandular (117, 46.2%), oropharyngeal (22, 8.7%), oculoglandular (3, 1.2%), pneumonic (25, 9.9%) and typhoidal 13, 5.1%). However, 13 (5.1%) patients presented with unusual clinical findings: prolonged fever of unknown origin (4 cases), otitis (2 cases), meningitis (2 cases), radiculitis (1 case), rhombencephalitis (1 case), acute pancreatitis (1 case), aortic aneurysm infection (1 case), and hip prosthesis infection (2 cases). These cases were diagnosed by the fortuitous isolation of a *F. tularensis* strain from blood cultures or cultures of deep suppurations. Typical risk factors for tularemia included: hunting (10 cases), farming (2), forester (1), contact with hares (28) or other game animals (5), hare meat consumption (8), tick bites (15), gardening (3), canyoning (1), and animal bites (4). Contact with a contaminated soil or vegetation were reported by 14 patients. One case was a laboratory worker who developed an ulceroglandular form of tularemia after manipulating *F. tularensis* cultures.
Over the past two decades, improved knowledge of tularaemia by physicians and better diagnostic methods have broadened the spectrum of clinical manifestations of this disease. A modern aspect concerns infections on prosthetic material that pose a new therapeutic challenge.

S5-4

Mutations targeting the FupA/B lipoprotein of F. tularensis LVS exposed to ciprofloxacin open a new way for Fluoroquinolone Resistance linked to OMV secretion and biofilm formation

Claire Siebert[1], Helena Lindgren[2], Sabrina Ferre[3], Corinne Villers[4], Sandrine Boisset[5], Julien Perard[6], Anders Sjosted[7], Max Maurin[8], Yohann Couté[9], Patricia Renesto[10]

[1]*TIMC-IMAG UMR 5525 - UGA CNRS, CHU Grenoble Alpes, Grenoble, France,* [2]*Umeå University, UMEÅ, Sweden,* [3]*UGA, CEA, Inserm, BIG-BGE, Grenoble, France,* [4]*Université de Caen Normandie, EA4655 U2RM, Caen, France,* [5]*Université Grenobles Alpes, Grenoble, France,* [6]*Univ. Grenoble Alpes, CNRS, CEA, BIG-LCBM, Grenoble, France,* [7]*Department of Clinical Microbiology, Bacteriology, and Laboratory for Molecular Infection Medicine Sweden, Umea, Sweden,* [8]*CHU Grenoble Alpes, Université Grenoble Alpes, Grenoble, France,* [9]*Univ. Grenoble Alpes, CEA, Inserm, BIG-BGE, Grenoble, France,* [10]*TIMC-IMAG UMR5525, CNRS-UGA, Grenoble, France*

Francisella tularensis is a Gram-negative bacteria responsible for the severe disease tularemia. While fluoroquinolones (FQ) represent a first-line alternative for treatment, the high prevalence of failure and relapses is a main concern. Functional DNA gyrase assays coupled with genome analysis of ciprofloxacin-resistant isolates of *F. tularensis* showed that acquisition of ciprofloxacin resistance is not exclusively related to DNA gyrase mutations. Besides such mutations, we observed that 80% of ciprofloxacin resistant strains of *F. tularensis* LVS generated upon a directed evolution experiment *in vitro* display a nonsense mutation in *fupA/B*. This observation, confirmed by others, led us to investigate whether FupA/B (Fer-Utilization Protein), a membrane protein responsible for siderophore-mediated iron acquisition and thought to be involved in bacterial virulence, was playing a role in ciprofloxacin resistance. We first evaluated the capacity of a resistant mutant derived from the evolution experiment to restore protein expression and ciprofloxacin susceptibility upon complementation. A mutant LVS-Δ*fupA/B* was also generated and complemented. In both cases a correlation between the ciprofloxacin minimal inhibition concentration (MIC) and FupA/B expression was observed, demonstrating the relationship between FupA/B expression and FQ resistance.

We then demonstrated using the virulent strain *F. tularensis* subsp. *tularensis* SCHU S4 that increased FQ susceptibility was dependent on *fupA* deletion whereas deletion of the gene encoding FupB has no effect. Interestingly, we obtained evidence that the deletion of the FupA/B lipoprotein promotes an increased secretion of outer membrane vesicles (OMVs). Mass spectrometry (MS)-based quantitative proteomic analyses identified with high confidence 801 different proteins in LVS and in LVS-Δ*fupA/B* OMVs including a subset of 23 proteins whose abundance is modified between both strains and which may therefore contribute to the observed increased FQ susceptibility. We obtained evidence that LVS-Δ*fupA/B,* which secretes more OMVs, also produces more biofilm than the LVS-wt strain. In conclusion, FupA/B deletion can thus be used by *Francisella* spp. as a new strategy to cope with FQ stress.

S5-5
Antimicrobial peptides against *Francisella tularensis*

Monique van Hoek[1], Stephanie Barksdale[2], Barney Bishop[2]
[1]George Mason University, Centreville, VA, [2]George Mason University, Manassas, VA

Antimicrobial peptides are produced as part of the innate immune response, and can have direct antibacterial activities as well as stimulate the host immune response. *Francisella*is a gram-negative gamma-proteobacterium that exhibits extreme resistance to the cyclic peptide antibiotic polymyxin B. However, antimicrobial peptides produced by the innate immune system of humans or other animals may be active against this organism. This would have implications not only for the host response to Francisella infection, but also for the potential development of those peptides into a potential therapeutic. We sought to identify antimicrobial peptides that would be effective against this Category A, Tier 1 biothreat agent to serve as a molecular platform for developing potential new therapeutics against this infection. We screened our extensive library of antimicrobial peptides against *Francisella novicida, Francisella tularensis* Live Vaccine Strain and *Francisella tularensis* SchuS4. From almost 200 peptides, we have identified only a few that exhibit significant antibacterial activity as measured by minimal inhibitory concentration (MIC) assays following the CLSI protocol. We were able to identify additional peptides that are antibacterial to *Francisella* under low ionic strength (EC_{50}) conditions. These lead peptide candidates will be described, and their activities compared and contrasted with their predicted structures and net charges. Finally, one antimicrobial peptide was tested in an *in vivo* murine pneumonic tularemia model and was able to rescue 60% of *Francisella*-infected mice when administered intraperitoneally.

S5-6
Identification of Potential Targets of Resazomycins, A Novel Family of Antibiotics Against *Francisella tularensis*

Deanna Schmitt[1], Michael Whaby[1], Jennifer Hickman[1], Devin Sindeldecker[1], Donald Primerano[1], James Denvir[2]
[1]West Liberty University, West Liberty, West Virginia, [2]Marshall University, Huntington, WV

Tularemia is a potentially fatal illness caused by the bacterium *Francisella tularensis*. Inhalation of less than 10 bacteria results in an acute pneumonia with an associated mortality rate of 30-60% if left untreated. Due to the potential use of *F. tularensis* as a weapon of bioterrorism and development of antibiotic resistance, new antibiotics are being sought against this pathogen. We have identified a novel family of resazurin-based compounds named resazomycins which exhibit antimicrobial activity against *F. tularensis* as well as the human pathogens *Neisseria gonorrhoeae* and *Helicobacter pylori*. In order to proceed with *in vivo* testing of resazomycins, their mechanism of action must be determined. To identify genes associated with resazomycin susceptibility, we cultivated *F. tularensis* on chocolate agar containing 20x the minimal inhibitory concentration of resazurin to select for resazomycin-resistant mutants. Whole genome sequencing was then performed on 48 resistant isolates and nonsynonymous mutations were identified in ten different protein-coding *F. tularensis* genes. Approximately 50% of the resazomycin-resistant isolates possessed mutations in FTL_1306 (*dipA*) and FTL_0959 (*pilD*). Therefore, we are currently investigating the role of *dipA* and *pilD* in *F. tularensis* susceptibility to resazomycins. We are also investigating whether genes induced in *F. tularensis* in the presence of resazurin are involved in inhibition of bacterial growth by resazomycins. Understanding the mechanism of action of resazomycins would facilitate further development of these compounds as potential treatments for tularemia.

S5-7
Complete genome sequences and comparative analysis of the novel pathogen *Francisella opportunistica*

Elizabeth Dietrich[1], Luke Kingry[1], Kiersten Kugeler[1], Craig Levy[2], Hayley Yaglom[3], Paul Mead[1], Jeannine Petersen[1]
[1]Centers for Disease Control and Prevention, Fort Collins, CO, [2]Maricopa County Department of Public Health, Phoenix, Canada, [3]Arizona Department of Health Services, Phoenix

Three human infections with the newly described species *Francisella opportunistica* have been identified. The organism was isolated from blood or cerebrospinal fluid of immune compromised individuals in Pennsylvania in 2005; in Massachusetts in 2006; and in Arizona in 2014. Only the isolate from Massachusetts has previously been genome sequenced. As *Francisella* species may be confused with *F. tularensis* in diagnostic assays, it is important to characterize additional potential pathogens in this genus. Here, we describe results from PacBio and Illumina genome sequencing for all three isolates in order to provide a comprehensive description of this novel organism. The three genomes display >99% average nucleotide identity (ANI) to each other and <90% ANI to any other *Francisella* genome, consistent with their designation as a novel species. The *F. opportunistica* genomes display approximately 87% ANI to *F. tularensis*. The most closely related species, at 89% ANI, is *F. persica*. Although the three genomes are collinear, they are extensively rearranged compared to genomes of other *Francisella* species. Several mobile elements are polymorphic among the three *F. opportunistica* isolates, including novel elements as well as elements with homology to previously described *Francisella* insertion sequence (IS) elements. All three genomes contain only a single copy of the *Francisella* pathogenicity island (FPI), which lacks the *pdpC* and *pdpE* genes. However, pseudogenized copies of *pdpC* and *pdpE* are found outside the FPI. This work confirms the discovery of closely related isolates of a novel opportunistic *Francisella* species from three unrelated clinical specimens. The public availability of genome sequences will aid in identification of further infections caused by this organism. Further work is needed to understand the epidemiology and ecology of this novel species.

Session 6

Epidemiology and Ecology

S6-1
Whole genome sequencing of *F. tularensis* from cultivable and non-cultivable sources to identify the origin of human cases of tularemia

Luke Kingry[1], Ryan Pappert[1], John Young[1], Laurel Respicio-Kingry[1], Jeannine Petersen[1]
[1]*Centers for Disease Control and Prevention, Fort Collins, CO*

Human cases of tularemia due to *F. tularensis* infection are rare in the United States, but can be of particular public health concern due to the severity of the illness, the complexity of subspecies and clades that cause illness and the pathogen's classification as a Tier 1 select agent. Human cases of tularemia are often preceded by epizootics in lagomorphs and rodents and thus investigations to determine sources of human exposure often rely on characterizing *F. tularensis* from animal carcasses. Using whole genome sequencing followed by whole genome multi-locus sequence typing (wgMLST), sequence similarity between epidemiologically linked strains can be investigated. We performed shotgun, paired-end sequencing followed by wgMLST to characterize >50 *F. tularensis* A2 strains from humans and animals from the United States, including epidemiologically linked strains from geographically localized outbreaks, epizootics, and human case investigations. For wgMLST analysis, 1,637 open reading frames were compared with >500 alleles identified across all genomes. This included sequencing of the bone marrow from two desiccated lagomorph carcasses to identify the source of a human infection. Resulting reads covered at least 99.9% of the *F. tularensis* genome, with 30X average coverage demonstrating the feasibility of this approach. *F. tularensis* DNA comprised 0.7 and 1.8% of the total DNA sequencing from the desiccated carcasses. wgMLST analysis showed that A2 strains without epidemiological links to each other displayed distinct allelic profiles. Similarly, strains from epizootics and case investigations were not identical when compared across short time frames and small geographic areas, consistent with a polyclonal origin of *F. tularensis* A2 strains during epizootics. Use of whole genome sequencing and wgMLST to characterize *F. tularensis* strains from both cultivable and non-cultivable sources furthers our understanding of sequence diversity among strains with and without epidemiological links which is critical for accurately identifying sources of human exposure.

S6-2
Whole-genome sequencing and molecular approaches for epidemiological surveillance and tracking of tularemia in France

Maëllys Kevin[1], Guillaume Girault[1], Moulay Ali Cherfa[1], Christiane Mendy[1], Yvan Caspar[2], Max Maurin[3], Claire Ponsart[1], Nora Madani[1]
[1]Université Paris-Est/ANSES, Laboratoire de Santé Animale, Maisons-Alfort, France, [2]Grenoble Alpes University Hospital, Grenoble, France, [3]CHU Grenoble Alpes, Université Grenoble Alpes, Grenoble, France

In France, tularemia, which is caused only by *Francisella tularensis* subsp. *holarctica*, is a sporadic disease affecting mainly wildlife animals and humans. *F. tularensis* species presents a low genetic diversity and remains poorly described in France. Over the last few years, whole-genome sequencing (WGS) appeared as the best approach to get an exhaustive overview of the genetic variations among isolates. The objective of this study was to describe the phylogenetic distribution of French isolates of *F. tularensis*, in order to develop a specific typing method using high-resolution melting (HRM) PCR technology. Indeed, identifying genomic features that are specific to sublineages is of great importance in epidemiology and public health. Whole genomes of around 250 French strains of human and animal origins from 1947 to 2018 were sequenced. A first classification using previously described canonical single nucleotide polymorphisms (canSNPs) was performed (Lärkeryd *et al.*, 2014). All isolates belonged to the B.44 clade –described in Western Europe– among twelve existing phylogenetic subclades. To go further in the resolution power, a whole-genome SNP analysis was carried out and around 700 SNPs were detected among all French isolates. We were able to accurately reconstruct the population structure according to the global phylogenetic framework and highlight eleven new subclades. Based on this global phylogeny, a molecular typing assay was designed for all genetic subclades identified in France. The HRM-PCR confirmed *in silico* results. This method enables rapid, accurate and inexpensive analysis of punctual mutations, such as SNPs. Genome-wide association studies however showed no relationships between genotypes and their geographical origin, year of isolation or host species.

In conclusion, we developed a rapid, reliable and cost-effective SNP-discriminative assay for molecular epidemiological surveillance of tularemia in France. Whole-genome SNP analysis also highlighted a large number of clusters among a single clade (B.44) in France, which reflects some diversity of French *F. tularensis* isolates within this clade. However, genotypes showed no correlation with geographical or temporal space or host species, which is consistent with the previously suggested hypothesis of a long-distance dispersal and slow replication rates with persistence in the environment (Dwibedi *et al.*, 2016).

S6-3
Genome-scale comparison of *Francisella tularensis* strains isolated in an endemic region of Spain.

Isabel Lopes de Carvalho[1], Miguel Pinto[2], Ruth Rodríguez-Pastor[3], Joana Isidro[2], Carolina Nunes[4], Francois Mougeot[5], Dolors Vidal[6], Juan José Luque-Larena[3], Raquel Escudero[7]

[1]National Institute of Health, Infectious Diseases Department, Águas de Moura, Portugal, [2]National Institute of Health, Lisboa, Portugal, [3]Dpto. Ciencias Agroforestales, Escuela Técnica Superior de Ingenierías Agrarias, Universidad de Valladolid; Instituto Universitario de Investigación en Gestión Forestal Sostenible, Palencia, Spain, [4]National Institute of Health, Águas de Moura, Portugal, [5]Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain, [6]Facultad de Medicina, Área Microbiología, Universidad de Castilla La Mancha, Ciudad Real, Spain, [7]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain

Background and aim: Tularemia is caused by the gram-negative intracellular pathogen *Francisella tularensis*. In Europe, the region of Castilla y León, Northwest Spain, is a major hotspot for tularemia, where the largest outbreaks of the disease have been recently reported. While rodents and lagomorphs are recognised as the main mammalian hosts in Europe, the common voles (*Microtus arvalis*) are documented key agents for human tularemia in Northwestern Spain, as evidenced by a spatial and temporal coincidence between human cases and increases in vole abundance. This study aimed to perform comparative genomics of *F. tularensis* isolates from tissue samples of *F. tularensis* positive voles with 11 human isolates from 2014, when an increased number of human cases was observed in the same area of Spain.

Material and Methods:

For this study, we selected thirty-four trapped voles, sampled in 80 km^2 of farmland in Palencia Province, Spain (42°1′N, 4°42′W), that tested Ft-positive, by conventional PCR and hybridization by reverse line blotting (targeting *lpn*A), and the multi-target TaqMan PCR, *tul4* and *ISFtu2* assays. Tissues (liver, spleen and lung) from each animal were minced, inoculated in chocolate agar PolyViteX™ at 37°C in 5% CO_2, and observed at 24, 48 and 72h post-inoculation. So far, after DNA extraction, four isolates, showing typical Ft growth, were subjected to paired-end whole-genome sequencing in an Illumina MiSeq apparatus, followed by genome assembly and bioinformatics analysis.

Results and conclusions:

All trapped voles tested positive for *F. tularensis* subsp. *holarctica*. A preliminary core-genome SNPs-based analysis, representing >99% of the genome, showed that all isolates (N=15) were distinguishable by 33 single nucleotide variant sites, representing two clear phylogenetic clusters. Noteworthy, four newly sequenced vole-isolated strains segregated together with five human-associated strains from 2014, suggesting the potential persistence of this strain in this region.

S6-4
Complete *Francisella* genomes generated by nanopore sequencing directly from human and animal clinical specimens

David Sundell[1], Andreas Sjödin[1], Jonas Näslund[1], Linda Karlsson[1], Catrine Lennartsson[1], Emelie Salomonsson[1], Anders Johansson[2], Mats Forsman[1]
[1]Swedish Defence Research Agency (FOI), Umeå, Sweden, [2]MIMS, Clinical Microbiology, Umeå University, Umeå, Sweden

Whole-genome sequencing of microbial pathogens in complex clinical samples is a promising approach for understanding microbial composition and predicting pathogen functions such as virulence and antibiotic resistance. The genome assembly of low abundant pathogens, however, is challenging due to the high levels of background DNA and limited amount of sequence reads. Here, we use selective whole genome amplification (SWGA) for amplification of microbial target genomes that are rare in complex backgrounds using short oligonucleotides (8-12 bases) scattered evenly across the genomes. We designed oligo sets optimized to five different *Francisella* species in two backgrounds (human and mouse). We selected the 24 most promising oligo pools to screen for *Francisella* sequences in serum of human or mouse origin. Each serum sample was spiked with 0.1% target bacteria. The samples were subjected to SWGA and we analyzed the resulting sequencing reads using a *Francisella* genus specific real-time PCR assay (GF1). Samples showing a minimum of 100 fold enrichment of target DNA compared to background were sequenced using MinION. The sequencing confirmed the successful enrichment of *Francisella* sequencing reads by SWGA from 0.1% to 50-70% of all reads in a sample. Subsequent direct assembly generated complete *Francisella* genomes. In ongoing work, we evaluate the method for use on spleen specimens from *Francisella*-infected animals and on ulcer specimens from humans with tularemia. The method described here for tularemia promises to open up a new era of diagnosing infection and characterizing causative microbes by generating complete microbial genomes from clinical specimens without the need of microbial culture.

S6-5
Type A.II strains of *Francisella tularensis* are quite capable of causing human disease and may be dispersed by wind and persist in the environmental in a quiescent state

Dawn Birdsell[1], Hayley Yaglom[2], Edwin Rodriguez[3], David Engelthaler[4], Matthew Maurer[5], Marlene Gaither[5], Jacob Vinocur[6], Joli Weiss[2], Joel Terriquez[6], Kenneth Komatsu[2], Mary Ormsby[5], Marette Gebhardt[5], Catherine Solomon[3], Linus Nienstadt[6], Charles Williamson[7], Jason Sahl[7], Paul Keim[7], **David Wagner**[8]
[1]Northern Arizona University, Flagstaff, AZ, [2]Arizona Department of Health Services, Phoenix, [3]Navajo County Public Health Services District, Show Low, [4]Translational Genomics Research Institute, Flagstaff, [5]Coconino County Public Health Services District, Flagstaff, [6]Northern Arizona Healthcare, Flagstaff, [7]Northern Arizona University, Flagstaff, [8]Northern Arizona University, FLAGSTAFF, UNITED STATES

Francisella tularensis subspecies *tularensis* (Type A) is divided into two distinct subgroups, A.I and A.II, that have little geographic overlap. A.II is found primarily in inter-mountain western North America, whereas A.I is found throughout the central-eastern regions and along the western coast. Observational human data and limited experimental mouse data suggest A.II is less virulent than Type B, which is in turn is less virulent than A.I. The five most recent tularemia cases from Arizona, including one fatality, were all caused by strains assigned to group A.II, demonstrating that A.II strains are quite capable of causing human disease. Indeed, only A.II strains have been isolated from humans and animals in Arizona to date. Comparisons of whole genome sequence (WGS) and geographic data from these five recent human cases and ten other A.II strains revealed interesting patterns. First, the recent cases were caused by strains from distinct clades within group A.II, suggesting distinct clades co-occur locally in the environment. Second, some closely-related strains are distant in geographic and temporal space, suggesting *F. tularensis* group A.II may be capable of long-distance dispersal, perhaps by wind. Third, these same isolates are also separated in temporal space, which is consistent with a very low evolutionary rate for A.II strains in the environment, perhaps due to persistence in a dormant state, such as the viable but non-culturable state that has been described for Type B. Consistent with a low evolutionary rate in the environment, the A.I and A.II groups appear to be highly monomorphic with much less genetic variation compared to Type B. We discovered just 309 SNPs among 14 A.II isolates separated by considerable geographic (maximum >1,000 km) and temporal (maximum 96 years) distances. In contrast, Type B exhibits much more diversity even across smaller geographic and temporal scales. These patterns suggest that, as has been suggested for Type B, strains from both groups A.I and group A.II may also persist long-term in the environment in a quiescent state where replication is non-existent or greatly arrested.

S6-6
Northern Trajectory of Human Tularemia — United States, 1965–2014

Natalie Kwit[1], Kiersten Kugeler[2], Christina Nelson[1], Paul Mead[1]
[1]Vermont Department of Health, Burlington, [2]Centers for Disease Control and Prevention, Fort Collins, CO

Background: Routes of human infection with *Francisella tularensis* are numerous, reflecting the complex ecology of the organism and factors contributing to its distribution, including arthropod vector and mammalian reservoir abundance. Although human infection occurs sporadically throughout the continental United States, historically, cases have been concentrated in the south central states of Arkansas, Louisiana, and Missouri. We used two methods to assess changes in the geographic distribution of human tularemia cases reported to CDC through the Nationally Notifiable Diseases Surveillance System during 1965–2014.

Methods: Human tularemia cases were geocoded to centroid coordinates for the county of patient residence. A linear model was fit comparing latitude and longitude of all cases by year, adjusted for population using annual, county-level U.S. Census estimates. To further evaluate geographic trend, a spatial scan cluster detection method based on log likelihood ratio [LLR] tests was used to identify the location of the highest risk spatial cluster of cases for 5-year moving average intervals.

Results: During 1965–2014, mean latitude of human tularemia cases in the lower 48 states moved 0.7 degrees North per decade ($P < .0001$), with no change in mean longitude ($P = .5$). Overall, spatial scan methods identified an area 668 km in diameter centered over northwestern Arkansas as the area of greatest risk (LLR: 6663; $P < .0001$). When evaluated in 5-year moving intervals, the average centroid latitude for this cluster moved from 35.8°N in 1965–1969 to 38.1°N in 2010–2014, a change of 256 km.

Conclusions: The distribution of human tularemia cases in the United States has moved progressively northward since 1965. This trajectory suggests areas of likely emergence where educational efforts may be most needed. The trend is independent of changes in population and may reflect shifts in environmental factors, human risk behaviors, or modes of transmission.

Author Index

Abate, Hiwot P2-20
Abazashvili, Natalia P1-40
Ackermann, Rahel P2-35
Adelani, Aanuoluwa P1-23, S2-5
Adler, Marlen P2-36, S3-5
Alam, Athar P2-39
Alcalay, Ron P1-31
Allen, Lee-Ann S3-1
Altpeter, Ekkehardt P2-35
Anderson, Paul P1-15
Annambhotla, Pallavi S5-2
Arnaboldi, Paul S2-4
Arroyo, Beatriz P1-14
Asad, Shadaba S5-2
Aslam, Saima S5-2
AuCoin, David P2-14
Bachert, Beth P1-24
Bakshi, Chandra S2-4, S4-5, P1-36
Balakhonov, Sergey P2-40, P1-39
Balonova, Lucie S3-4
Balzano, Phillip P1-08
Bar-Haim, Erez P1-28
Bar-On, Liat P1-30, P1-28
Barksdale, Stephanie S5-5
Barry, Eileen P2-01, P1-08, P1-03, P2-33
Basavaraju, Sridhar S5-2
Basler, Marek P2-38, S4-1
Bavlovič, Jan P1-25, P1-02
Benaoudia, Sacha S1-4
Benuchova, Milota S1-1
Benziger, Peter P2-37, S3-6
Beuret, Christian P2-35
Bhargava, Varunika P2-03
Biot, Fabrice P1-24
Birdsell, Dawn S6-5, P1-29, P2-16
Bishop, Barney S5-5
Boan, Daniel P1-15
Boisset, Sandrine S5-4, S5-3
Bosio, Catharine P2-25, S1-3, S1-6
Bowling, Jennifer P1-03, P2-01, P2-33, S2-1
Bozue, Joel P1-20, P1-09, P1-24
Brachveli, Gvantsa P1-40
Bradford, Kate S1-5
Brodmann, Maj P2-38, S4-1
Brook, Helen P2-03, S2-2
Brown, Jennifer S5-2
Broz, Petr S4-1, P2-38
Brudal, Espen P2-34
Buntyn, Robert S1-3
Burjanadze, Irma P1-40
Burns, Kevin S5-2
Cantlay, Stuart S3-7, P1-10
Carifo, Karen S5-2
Carlson, Bradley S1-7
Caroline, Amy P2-33
Caspar, Yvan P1-11, S6-2, S5-3
Casulli, Joshua S1-2, P1-13
Celli, Jean S3-5, P2-36
Chakhunashvili, Giorgi P2-30
Chanturia, Gvantsa P1-40
Charbit, Alain S1-8
Cherfa, Moulay Ali S6-2
Chitlaru, Theodor P1-28, P1-30
Chu, Ping S2-5
Clark, Graeme P1-13, S1-2
Cobb, Ronald S2-7
Cohen, Hila P1-31, P1-30, P1-28
Cohen, Ofer P1-31, P1-30, P1-28
Collins, Elliot P1-18 , P1-04
Conlan, Wayne S2-2, K-1, P1-12
Couté, Yohann S5-4
Crane, Deborah S1-6
D'Elia, Riccardo S1-2, P1-13
Dagher, Hikmat S5-2
Dankova, Vera S3-4, P2-27
Daye, Sharon P1-09
De Pascalis, Roberto P1-34, S2-2, S2-6, P2-02
DeMers, Haley P2-14

Denvir, James S5-6
Dewan, Kalyan S1-7, P2-06
Dietrich, Elizabeth S5-7
Dominguez, Sedelia P1-05
Dove, Simon P2-05
Dow, Joshua S1-2
Dreier, Roland P2-38, S4-1
Elia, Uri P1-30, P1-31, P1-28
Elkins, Karen P2-02, P1-35, S1-5, P2-09, S2-2, P1-34, P2-03, S2-6
Eneslätt, Kjell P2-11
Engelthaler, David S6-5
Epstein, Eyal P1-31
Escudero, Raquel S6-3, P1-14, P1-19
Eshraghi, Aria S3-5, P2-36
Faber, Mirko P1-33
Fabrik, Ivo P2-21
Fabrikova, Daniela P2-21
Felix, Jan S4-4
Ferre, Sabrina S5-4
Fife, Mark S1-2
Flick-Smith, Helen S2-3
Foreman, Amanda P2-28, P1-06
Forsman, Mats P2-23, P1-29, S6-4
Fox, Todd P1-41
Frey, Blake S2-2
Fritz, Curtis S5-2
Gaither, Marlene S6-5
Gates-Hollingsworth, Marcellene P2-14
Gebhardt, Marette S6-5
Geleishvili, Marika P2-30
Gelhaus, Herbert Carl P2-09, P1-15, S2-2, P2-11, P1-12
Giorgidze, Gocha P2-30
Girault, Guillaume S6-2
Goldapel , Eduard P2-40
Golovliov, Igor P2-39, P2-26, P2-11
Gonzalez, Anthony S5-2
González Martín-Niño, Rosa P1-14
Goralski, Tyler P1-38, S4-2
Granberg, Malin P1-29
Griffiths, Gareth P2-34
Grub, Carola P1-29
Grunow, Roland P2-04, P2-35, P1-37, P1-33, S5-1, P1-17, P1-01
Gunn, John P1-32
Gutsche, Irina S4-4
Gygli, Sebastian P2-35
Gyuranecz, Miklós P1-19
Habus, Josipa P1-26
Haldemann, Berit S5-1, P1-37
Ham, John S5-2
Hannah, Emily P2-14
Harrison, Harrison P1-32
Hau, Derrick P2-14
Hazlett, Karsten P2-33, P2-01, P1-42, P1-03
Healy, James P1-18
Helland, Sandra P1-29
Helsel, Elizabeth S5-2
Hemmilä, Heidi P1-29
Henry, Thomas S1-4, P2-18, P1-07
Hercik, Kamil P2-29
Hernandez, Ana P2-15
Heuner, Klaus P1-33, P1-17, S5-1, P1-01, P1-37
Hickman, Jennifer S5-6
Hinckley, Alison S5-2
Hoang, Ky P1-32
Horzempa, Joseph P1-04, S3-7, P1-18, P2-15, P1-10
House, Robert S2-7
Houston, Stephanie S1-2
Huntley, Jason S3-3, S3-2
Hussell, Tracy S1-2, P1-13
Ii, Alexandra P2-31
Imnadze, Paata P1-40, P2-30
Ireland, Robin P2-25, S1-6
Isidro, Joana S6-3
Izoria, Mariam P2-30
Jacob, Daniela P1-01, S5-1, P2-04, P2-35, P1-33, P1-37
Jacobs, MacKenzie P1-18
Jado, Isabel P1-14
Janowski, Ann S3-1
Javed, Eram P2-39
Jessop, Forrest S1-3, P2-25

Johansson, Anders P1-19, S6-4, P1-29
Jones, Jefferson S5-2
Jubete, Fernando P1-14
Karlsson, Linda S6-4
Karrer, Urs P2-35
Katkere, Bhuvana S1-7, P2-06
Kawula, Thomas S3-5, P1-06, P2-36, P1-20, P1-05, P2-28
Keiler, Kenneth P1-38, S4-2
Keim, Paul S6-5
Kelley, Walter S5-2
Kelly, Katherine P2-36, S3-5
Kevin, Maëllys S6-2
Khmaladze, Ekaterine P1-40
Kijek, Todd P1-09, P1-20
King, Kayla S4-5, P1-36
Kingry, Luke S5-2, S5-7, S6-1
Kinori, Adi P1-28
Kirimanjeswara, Girish P1-38, S1-7, S4-2, P2-06
Klimentova, Jana P1-25, P1-02
Klose, Karl S2-5, P1-23
Koeppen, Kristin P1-01, P1-37, P1-17, S5-1
Komatsu, Kenneth S6-5
Kopeckova, Monika P2-07
Kopping, Erik P2-37, S3-6, P2-08
Korneva, Aleksandra P2-40
Koroleva, Galina P1-24
Kotaria, Nato P1-40
Krafft, Melissa S5-2
Krasnykov, Kyrylo S1-4
Kratka, Lucie P2-29
Krocova, Zuzana P2-21, P2-17, S1-1
Kubelkova, Klara P2-17, S1-1, P1-27, P1-25
Kugeler, Kiersten P2-10, S6-6, S5-7
Kulikalova, Elena P1-39, P2-40
Kurolt, Ivan P1-26
Kurtz, Sherry P1-34, S2-6, P1-35, P2-09, P2-03
Kwit, Natalie S6-6, S5-2, P2-10
Ladner, Jason P1-24
Lagos, Leidy P2-34
Lagrange, Brice S1-4
Lampe, Elisabeth P2-34
Lays, Claire P2-18
Ledvina, Hannah S3-5, P2-36
Lee, Brian S3-5, P2-36
Lenco, Juraj P2-29, P1-27, P2-18
Lennartsson, Catrine S6-4
Levitz, Stuart S2-3
Levy, Craig S5-7
Lindgren, Helena P1-07, P2-11, S5-4
Lindgren, Petter P2-23
Link, Marek S3-4, P2-21
Liu, Qing P1-12
Lopes de Carvalho, Isabel P2-12, S6-3
Lovett, Sean P1-24
Lu, Jinxing P2-19
Lundmark, Eva P2-23
Luque-Larena, Juan José P1-14, S6-3
Ma, Zhuo P1-36, S4-5
Macela, Ales P2-17, S1-1
Madani, Nora S6-2
Malakmadze, Naile P2-30
Malik, Meenakshi S2-4, S4-5, P1-36
Mann, Barbara P2-13, P2-20
Mansour, Ahd S2-4
Marakasova, Ekaterina P2-31
Marecic, Valentina P2-24, P1-26
Margaletic, Josip P1-26
Markley, Rachel S1-7, P2-06
Markotic, Alemka P1-26
Markov, Evgenii P2-40
Matovina, Mihaela P1-26
Maurer, Matthew S6-5
Maurin, Max S6-2, S5-3, P1-11, S5-4
Maxaradze, Maia P2-30
Mazepa, Andrey P2-40, P1-39
Mazor, Ohad P1-31
McCormick, Alison S2-4
McCracken, Jenna S3-1
Mead, Paul S6-6, P2-10, S5-7
Mechaly, Adva P1-31
Medina-Garcia, Luis S5-2
Mendy, Christiane S6-2

Merz, Alexey P2-36, S3-5
Mihelcic, Mirna P1-26, P2-24
Mohammadi, Nasibeh P1-07
Mohapatra, Nrusingh P1-32
Mohler, Kelli S5-2
Moreau, Brett P2-13
Mou, Sherry P1-24, P1-09
Mougeot, Francois S6-3, P1-14
Mougous, Joseph P2-36, S3-5
Munson, Robert P1-32
Murua, Christian S5-2
Myrtennäs, Kerstin P1-29, P2-16, P1-19
Nardone, Glenn P2-25
Nascimento, Paula P2-12
Näslund, Jonas S6-4
Nau, Gerard P2-15
Nelson, Christina P2-10, S6-6, S5-2
Nelson, Kristina P2-31
Nevins, Cheryl P1-15
Nguyen, Jesse S2-5, P1-23
Nienstadt, Linus S6-5
Nigou, Jérôme S1-8
Nikkari, Simo P1-29
Nikolaev, Valerii P2-40
Nora, Valerija P2-24
Nualnoi, Teerapat P2-14
Núncio, Maria P2-12
Nunes, Carolina S6-3
Öhrman, Caroline P2-16
Ormsby, Mary S6-5
Ostroff, Gary S2-3
Oyston, Petra S2-3
Ozanic, Mateja P1-26, P2-24
Ozsurekci, Yasemin P2-14
Palacios, Gustavo P1-24
Pandit, Sujata P2-14
Pantsulaia, Meri P1-40
Pappert, Ryan S6-1
Pavkova, Ivona P2-21, P2-07, P1-27, P1-25
Pelkonen, K P1-29
Pelloux, Isabelle S5-3
Peng, Yao P2-19
Perard, Julien S5-4
Perevalova, Maria P1-39
Pérez, Natalie P1-41
Petersen, Jeannine S6-1, S5-7, S5-2
Peterson, Snow P2-36, S3-5
Pflughoeft, Kathryn P2-14
Philimonenko, Vlada P2-27
Pilo, Paola P2-35
Pinkas, Dominik P2-27
Pinto, Miguel S6-3
Plemel, Rachael S3-5, P2-36
Plzakova, Lenka S1-1, P1-25, P2-21, P2-17
Ponsart, Claire S6-2
Poupore, Amy P2-02
Prabhu, K Sandeep S1-7
Prandi, Jacques S1-8
Primerano, Donald S5-6
Proksova, Magdalena P2-18
Qin, Aiping P2-19, P2-20
Radonić, Aleksander P1-37, S5-1
Ramakrishnan, Girija P1-41, P2-20
Ramsey, Kathryn P2-05
Rashid, Maheen S4-3
Reed, Doug P1-03, P2-01, P2-33, S2-1
Rehulka, Pavel P2-21, P2-07, P2-18
Rehulkova, Helena P2-18, P2-29, P2-07
Ren, Guoping S3-3
Renesto, Patricia S5-3, S5-4, P2-22, S4-4
Respicio-Kingry, Laurel S5-2, S6-1
Robert, Camille S1-8
Roberts, Lydia S1-6
Rodriguez, Edwin S6-5
Rodriguez, Sabrina P1-24
Rodríguez-Pastor, Ruth S6-3, P1-14
Rohleder, Anna P2-04
Rossi, Stefano S1-2
Rotem, Shahar P1-28, P1-30
Rydzewski, Kerstin P1-17
Sahl, Jason S6-5
Salomonsson, Emelie P2-23, S6-4
Sampath, Vinaya S4-3
Sandra, Appelt P2-04

Šantić, Marina P1-26, P2-21, P2-27, P2-24
Schmitt, Deanna S5-6, P1-04, S3-7
Schröder, Wolfgang P1-07
Schuerch, Nadia P2-35
Schwartz, Amy P2-10
Schwartz, Justin S3-1
Schwarz, Benji P2-25, S1-3
Seeber, Roger P1-18
Senitkova, Iva P2-27
Sesko (Sheshko), Valeria S3-4, P2-26
Shavishvili, Merab P1-40
Shay, Ashley S1-7
Siebert, Claire S4-4, P2-22, S5-4
Simonyan, Vahan P1-34
Sindeldecker, Devin S5-6
Sissonen, Susanna P1-29
Sjödin, Andreas S6-4, P2-23
Sjöstedt, Anders P1-07, P2-11, P2-34, S2-2, P1-12, P2-39, S1-4, S5-4
Skerrett, Shawn S3-5, P2-36
Solomon, Catherine S6-5
Sosna, Bill P1-15
Spidlova, Petra P2-27, P2-29, P1-27
Steele, Shaun P1-05, P2-36, P2-34, P1-20, P1-06, P2-28, S3-5
Stinson , Elizabeth P1-03, P2-01, P2-33
Stojkova, Pavla P2-29, P2-27
Stulik, Jiri P2-18, P2-26, P2-27, P2-29, P1-25, P1-27, P2-21, S3-4, P1-02
Sukhiashvili, Roena P1-40
Sumner, Sarah S1-7
Sundell, David P2-16, S6-4
Svoboda, Petra P1-26
Syngeeva, Ayuna P2-40, P1-39
Tallo, Thomas P2-05
Tandberg, Julia P2-34
Terriquez, Joel S6-5
Tevdoradze, Tea P1-40
Thanassi, David S3-6, P2-08, S4-3, P2-37
Thelaus, Johanna P2-23
Thorkildson, Peter P2-14
Tlapák, Hana P1-17
Tonteri, Elina P1-29
Toothman, Ronald P1-24
Toscano Rico, Miguel P2-12
Touron, Sonia S1-8
Travis, Mark S1-2, P1-13
Tsertsvadze, Nikoloz P1-40
Tsertsvadze, Nikoloz P2-30
Tsilosani, Maka P2-30
Tummillo, Kristen P1-42
Turk, Nenad P1-26
Ulanova, Lilia P2-34
van Hoek, Monique P2-31, S5-5, P1-21, P1-22
Vercellone, Alain S1-8
Vidal, Dolors P1-14, S6-3
Villers, Corinne P2-22, S5-4
Vinocur, Jacob S6-5
Voskanian-Kordi, Alin P1-34
Vozandychová, Věra P2-21
Vucelja, Marko P1-26
Wagner, David P1-29, S6-5
Wallet, Pierre S1-4
Wang, Yanhua P2-19
Wehrly, Tara S1-6
Weiss, Joli S6-5
Whaby, Michael S5-6
Whelan, Adam S2-3
Wiggins, Landon S5-2
Willett - O'Malley, Katherine S2-1, P1-03, P2-01, P2-33
Williamson, Charles S6-5
Williamson, David S1-7, P2-06
Williamson, Diane P1-13, S1-2
Winther-Larsen, Hanne P2-34
Wittwer, Matthias P2-35
Wolf, Timo P1-37, S5-1
Worden, Madeline S4-5, P1-36
Worsham, Patricia P1-09, P1-24
Yaglom, Hayley S5-7, S6-5
Young, John S6-1
Zakalashvili, Mariam P1-40
Zakhashvili, Khatuna P2-30
Zanger, Philipp P1-37, S5-1
Zellner, Briana S3-2, S3-3
Zhang, Ying S5-2
Zhghenti, Ekaterine P1-40
Zogaj, Xhavit S2-5, P1-23

Attendee List

Aanuoluwa A Adelani
University of Texas at San Antonio
One UTSA Circle
University of Texas at San Antonio, TX
San Antonio, TX 78249 United States
E-mail: aanuadelani@gmail.com

Lee-Ann H Allen
University of Iowa
431 Newton Rd
505 EMRB
Iowa City, IA 52242 United States
Tel. 319.335.4258
E-mail: lee-ann-allen@uiowa.edu

Bernard P. Arulanandam
University of Texas at San Antonio
1 UTSA Circle
San Antonio, TX 78249 United States
Tel. 210.458.8176
E-mail: bernard.arulanandam@utsa.edu

Beth Bachert
United States Army Medical Research
Institute of Infectious Diseases
Frederick, MD United States
E-mail: beth.a.bachert.ctr@mail.mil

Chandra Shekhar Bakshi
New York Medical College
15 Dana Road
Valhalla, NY 12077 United States
Tel. 914.594.4814
E-mail: Shekhar_Bakshi@nymc.edu

Liat Bar-On
Israel Institute for Biological Research
PO Box 19, Ness-Ziona 74100, Israel
E-mail: liatgree@yahoo.com

Eileen Barry
University of Maryland at Baltimore
685 West Baltimore Street
HSF1 480
Baltimore, MD 21201 United States
Tel. 410.706.3702
E-mail: embarry@som.umaryland.edu

Jan Bavlovič
University of Defense, Faculty of Mili-
tary Health Sciences
Trebesska 1575
Hradec Kralove, Česká Republika 500
01 Czech Republic
E-mail: jan.bavlovic@unob.cz

Peter Todd Benziger
Stony Brook University
341 Pond Path
East Setauket, NY 11733 United States
Tel. 215.595.4240
E-mail: peter.benziger@stonybrook.edu

Varunika Bhargava
CBER/FDA
10903 New Hampshire Avenue
Building 52/72, Rm 5326
Silver Spring, MD 20993 United States
E-mail: Varunika.Bhargava@fda.hhs.gov

Dawn N Birdsell
Northern Arizona University
1395 South Knoles Drive, Building 56
PO Box 4073
Flagstaff, AZ 86011 United States
Tel. (928) 523-0552
E-mail: Dawn.Birdsell@nau.edu

Sandrine Boisset
Université Grenobles Alpes
Boulevard de la chantourne
Grenoble, 38 043 France
E-mail: sboisset@chu-grenoble.fr

Catharine Bosio
Rocky Mountain Laboratories
903 S 4th Street
Hamilton, MT 59840 United States
E-mail: bosioc@niaid.nih.gov

Talar Boskani
The Public Health Agency of Sweden
Nobelsväg 18
Stockholm, Sweden 17182 Sweden
Tel. 0046723705679
E-mail: talar.boskani@folkhalsomyndigheten.se

Jennifer Bowling
University of Pittsburgh
3501 Fifth Ave
Pittsburgh, PA 15260 United States
E-mail: jeb263@pitt.edu

Joel A. Bozue
USAMRIID
1425 Porter Street
Bacteriology Division
Ft Detrick, MD 21702 United States
Tel. 301.619.4934
E-mail: joel.a.bozue.civ@mail.mil

Maj Brodmann
Biozentrum, University of Basel
Klingelbergstrasse 50/70
Basel, 4056 Switzerland
E-mail: maj.brodmann@unibas.ch

Stuart Cantlay
West Liberty University
West Liberty, WV United States
E-mail: stuart.cantlay@westliberty.edu

Yvan Caspar
Grenoble Alpes University Hospital
Laboratoire de bactériologie, Institut de Biologie et Pathologie, CHU Grenoble Alpes CS10217
Grenoble, 38043 France
E-mail: YCaspar@chu-grenoble.fr

Joshua Casulli
University of Manchester
2.14b Core Technology Facility
Grafton Street
Manchester, M13 9NT United Kingdom
Tel. 07920264439
E-mail: joshua.casulli@postgrad.manchester.ac.uk

Gvantsa Chanturia
National Center for Disease Control and Public Health (NCDC)
Richard G. Lugar Center for Public Health Research
9 M. Asatiani St
Tbilisi, 0177 Georgia
Tel. +995591228507
E-mail: gvantsa.chanturia@ncdc.ge

Theodor Chitlaru
IIBR
PO Box 19
Ness-Ziona, 75100 Israel
E-mail: theo.chitlaru@gmail.com

Ofer Cohen
IIBR
Ness-Ziona
Ness-Ziona, 74100 Israel
E-mail: ofercohen777@gmail.com

Hila Cohen
IIBR
PO Box 19
Ness-Ziona, 74100 Israel
E-mail: hilulimcohen@gmail.com

Elliot Collins
West Liberty University
208 University Dr
CUB 139
West Liberty, WV 26074 United States
E-mail: emcollins@westliberty.edu

Wayne Conlan
National Research Council Canada
100 Sussex Drive
Ottawa, Ontario K1C 2M7 Canada
Tel. 613.993.8829
E-mail: wayne.conlan@nrc-cnrc.gc.ca

Riccardo D'Elia
Defence Science and Technology Laboratory
Porton Down
Salisbury, SP4 0JQ United Kingdom
E-mail: rvdelia@dstl.gov.uk

Roberto De Pascalis
CBER/FDA
10903 New Hampshire Avenue
Silver Spring, MD 20903 United States
E-mail: roberto.depascalis@fda.hhs.gov

Elizabeth A Dietrich
Centers for Disease Control and Prevention
3156 Rampart Rd.
Fort Collins, CO 80521 United States
E-mail: wul2@cdc.gov

Sedelia Dominguez
Washington State University
WSU Paul G. Allen School for Global Animal Health Allen Center
PO Box 647090
Pullman, WA 99163 United States
E-mail: sedelia.dominguez@wsu.edu

Simon Dove
Boston Childrens Hospital, Harvard Medical School
300 Longwood Avenue Enders 761.1
Boston, MA 02115 United States
E-mail: Simon.Dove@childrens.harvard.edu

Uri Elia
IIBR
Ness-Ziona, Israel
E-mail: eliauri23@gmail.com

Karen L. Elkins
CBER/FDA
10903 New Hampshire Ave
Building 52/72, Room 5330
Silver Spring, MD 20993 United States
Tel. 01.240.402.9507
E-mail: karen.elkins@fda.hhs.gov

Kjell Peter Eneslätt
Umeå University
Clinical Microbiology
Umeå, 90187 Sweden
Tel. +46 90 785 46 59
E-mail: kjell.eneslatt@umu.se

Raquel Escudero
Centro Nacional de Microbiología, Instituto de Salud Carlos III
CTRA. MAJADAHONDA-POZUELO KM 2
Majadahonda, Madrid 28220 Spain
Tel. 34 91 8223953
E-mail: rescude@isciii.es

Amanda Leigh Foreman
Paul G. Allen School for Global Animal Health
Washington State University
Pullman, WA 99164 United States
E-mail: amanda.foreman@wsu.edu

Mats Forsman
Swedish Defence Research Agency
Umeå, Sweden
E-mail: mats.forsman@foi.se

Herbert Carl Gelhaus
MRI Global
425 Volker Blvd
Kansas City, MO 64110 United States
E-mail: cgelhaus@mriglobal.org

Eduard Goldapel
Irkutsk Antiplague Research Institute of Siberia and Far East
Trilisser str. 78
Irkutsk, 664047 Russian Federation
Tel. +79086465977
E-mail: tapuz82@mail.ru

Tyler D Goralski
The Pennsylvania State University
State College, PA United States
E-mail: tdg143@psu.edu

Roland Grunow
Robert Koch Institute
Seestr. 10
Berlin, 13353 Germany
E-mail: grunowr@rki.de

John Gunn
The Ohio State University
Biomedical Research Tower rm 794
460 W 12th Ave
Columbus, OH 43210 United States
E-mail: john.gunn@osumc.edu

Emily Hannah
University of Nevada, Reno
Reno, NV United States
E-mail: em.hannah93@gmail.com

Karsten R.O. Hazlett
Albany Medical College
47 New Scotland Ave.
Albany, NY 12208 United States
Tel. 518.262.2338
E-mail: Hazlett@mail.amc.edu

Sandra Yaneth Helland
Norwegian Armed Forces Medical Services
Skogmotoppen 29 H
JESSHEIM, Ullensaker 2053 Norway
Tel. 4747475070
E-mail: helland.sandra@gmail.com

Thomas Henry
CIRI, International Center for Infectiology Research
Inserm U1111, CNRS, UMR5308
21 avenue Tony Garnier
Lyon, 69007 France
E-mail: thomas.henry@inserm.fr

Kamil Hercik
University of Defence, Faculty of Health Sciences
Hradec Kralove, Czech Republic
E-mail: kamil.hercik@unob.cz

Klaus Heuner
Robert Koch Institute
Berlin, Germany
E-mail: heunerk@rki.de

Ky V Hoang
The Ohio State University
460 W 12th Ave
Columbus, OH 43210 United States
Tel. 614.598.3935
E-mail: kythao2012@gmail.com

Joseph Horzempa
West Liberty University
208 University Drive
CUB 139
West Liberty, WV 26074 United States
Tel. 1-304.336.8284
E-mail: joseph.horzempa@westliberty.edu

Robert V House
Ology Bioservices
8490 Progress Drive
Suite 150
Frederick, MD 21701 United States
Tel. 301.276.7851
E-mail: robert.house@ologybio.com

Jason Frederick Huntley
University of Toledo
3000 Arlington Avenue-HEB200D
University of Toledo-HSC
Toledo, OH 43614-2598 United States
Tel. 419.383.5456
E-mail: Jason.Huntley@utoledo.edu

Daniela Jacob
Robert Koch Institute
Seestrasse 10
Berlin, 13353 Germany
Tel. +49 30 18754 2934
E-mail: Jacobd@rki.de

MacKenzie Lynn Jacobs
West Liberty University
311 Porter Street
New Cumberland, WV 26047 United States
Tel. 304.914.7089
E-mail: mljacobs@westliberty.edu

Forrest Jessop
NIAID
903 S 4th St
Hamilton, MT 59840 United States
Tel. 406.802.6094
E-mail: forrest.jessop@nih.gov

Thomas Hardin Kawula
Paul G. Allen School for Global Animal Health
Washington State University
PO Box 647090
Pullman, WA 99164 United States
Tel. 509.335.1301
E-mail: tom.kawula@wsu.edu

Maëllys Kevin
Université Paris-Est/ANSES, Laboratoire de Santé Animale
22 rue Pierre et Marie Curie
Maisons-Alfort, 94700 France
E-mail: maellys.kevin.ext@anses.fr

Hamda Khan
United States Food and Drug Administration
10903 New Hampshire Ave, Building 52/72, Room 5326
Silver Spring, MD 20903 United States
E-mail: hamda.khan@fda.hhs.gov

Todd Kijek
USAMRIID
1425 Porter Street
Ft. Detrick, MD 21702 United States
E-mail: todd.m.kijek.mil@mail.mil

Kayla A King
ACPHS
106 New Scotland Ave
Albany, NY 12208 United States
Tel. 413.348.7344
E-mail: kaylaaaking@gmail.com

Luke C Kingry
Centers for Disease Control and Prevention
3156 Rampart Rd
Fort Collins, CO 80521 United States
E-mail: vtx8@cdc.gov

Girish Soorappa Kirimanjeswara
The Pennsylvania State University
W205 Millennium Science Complex
University Park, PA 16802 United States
Tel. 814.863.5250
E-mail: gsk125@psu.edu

Jana Klimentova
University of Defense, Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove, 500 01 Czech Republic
E-mail: jana.klimentova@unob.cz

Karl E. Klose
University of Texas San Antonio
One UTSA Circle
South Texas Center for Emerging Infectious Diseases
San Antonio, TX 78249 United States
Tel. 210.458.6140
E-mail: Karl.Klose@utsa.edu

Kristin Koeppen
Robert Koch Institute
Seestr. 10
Berlin, 13353 Germany
E-mail: koeppenk@rki.de

Monika Kopeckova
Faculty of Military Health Sciences, University of Defence
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: monika.kopeckova@unob.cz

Erik Kopping
Stony Brook University
Center for Infectious Diseases
295 CMM Building
Stony Brook, NY 11794-5120 United States
Tel. 631.632.4231
E-mail: erik.kopping@stonybrook.edu

Zuzana Krocova
FMHS UoD
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: zuzana.krocova@unob.cz

Klara Kubelkova
University of Defence, Faculty of Military Health Sciences
1575 Trebesska
Hradec Kralove, Kralovehradecky 50011 Czech Republic
Tel. 973255193
E-mail: klara.kubelkova@unob.cz

Kiersten J Kugeler
Centers for Disease Control and Prevention
3156 Rampart Road
Fort Collins, CO 80525 United States
E-mail: bio1@cdc.gov

Sherry Kurtz
CBER/FDA
10903 New Hampshire Ave
Bldg 52/72 Rm 5328
Silver Spring, MD 20903 United States
Tel. 240.402.9655
E-mail: sherry.kurtz@fda.hhs.gov

Natalie Kwit
Vermont Department of Health
108 Cherry Street; Suite 304
Burlington, VT 05401 United States
E-mail: natalie.kwit@vermont.gov

Marilynn A. Larson
University of Nebraska Medical Center
985900 Nebraska Medical Center
Omaha, NE 68198-5900 United States
Tel. 402.559.9115
E-mail: malarson@unmc.edu

Hannah Elizabeth Ledvina
Department of Microbiology, University of Washington School of Medicine
1705 NE Pacific St
J-135
Seattle, WA 98195 United States
Tel. 206.685.6740
E-mail: hledvina@uw.edu

Chelsea Lehman
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Building 52/72 Rm 5348
Silver Spring, MD 20903 United States
E-mail: chelsea.lehman@fda.hhs.gov

Helena Lindgren
Umeå University
BLOMSTERVÄGEN 6
UMEÅ, Vasterbotten 90352 Sweden
Tel. 0702294582
E-mail: helena.e.lindgren@umu.se

Isabel Lopes de Carvalho
National Institute of Health, Infectious Diseases Department
Avenida da Liberdade, 5
Águas de Moura, 2965-575 Portugal
Tel. +351919042474
E-mail: isabel.carvalho@insa.min-saude.pt

Meenakshi Malik
Albany College of Pharmacy and Health Sciences
106 New Scotland Avenue
Albany, NY 12208 United States
Tel. 518.694.7168
E-mail: Meenakshi.Malik@acphs.edu

Barbara Mann
University of Virginia
345 Crispell Dr
PO BOX 801340
Charlottesville, VA 22908 United States
Tel. 434.924.9666
E-mail: bjm2r@virginia.edu

Max Maurin
CHU Grenoble Alpes, Université Grenoble Alpes
Bd Chantourne
Institut de Biologie et Pathologie
Grenoble, 38043 France
E-mail: mmaurin@chu-grenoble.fr

Mirna Mihelcic
Faculty of Medicine, University of Rijeka
Brace Branchetta 20
Rijeka, Primorsko-Goranska 51 000
Croatia
Tel. +38551651144
E-mail: mirna.mihelcic@medri.uniri.hr

Nasibeh Mohammadi
Umeå University
LINNEAUS VÄG 10
KEMISKA INST.
Umeå, 90 187 Sweden
E-mail: nasibeh.mohammadi@umu.se

Kerstin Myrtennäs
Swedish Defence Research Agency (FOI)
Cementvägen 20
Umeå, 90182 Sweden
E-mail: bioinformatics@foi.se

Gerard Joseph Nau
Alpert School of Medicine at Brown University
593 Eddy Street
Aldrich 722
Providence, RI 02903 United States
Tel. 401.444.7432
E-mail: gerard_nau@brown.edu

Christina Nelson
CDC
3156 Rampart Rd. P-02
Fort Collins, CO 80521 United States
Tel. 970.225.4259
E-mail: wje1@cdc.gov

Jérôme Nigou
Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS
BP64182, 205 route de Narbonne
Toulouse, 31077 France
E-mail: jerome.nigou@ipbs.fr

Caroline Öhrman
Swedish Defence Research Agency (FOI)
Cementvägen 20
Umeå, 90182 Sweden
Tel. +46725709230
E-mail: caroline.ohrman@foi.se

Petra C.F. Oyston
DSTL
Microbiology, B07 Room 201
DSTL Porton Down
Salisbury, Wiltshire SP4 0JQ United Kingdom
Tel. +44 1980 957801
E-mail: pcoyston@dstl.gov.uk

Jason Michael Park
Washington State University
WSU Paul G. Allen School for Global Animal Health Allen Center
PO Box 647090 1155 College Ave.
Pullman, WA 99164 United States
Tel. 979.571.2204
E-mail: jpark12@wsu.edu

Ivona Pavkova
University of Defence, Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove, 500 02 Czech Republic
E-mail: ivona.pavkova@unob.cz

Jeannine M Petersen
Centers for Disease Control and Prevention
3156 Rampart Road
Fort Collins, CO 80521 United States
E-mail: nzp0@cdc.gov

Lenka Plzakova
University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: lenka.plzakova@unob.cz

Magdalena Proksova
University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: magdalena.proksova@unob.cz

Girija Ramakrishnan
University of Virginia
Department of Medicine/Infectious Diseases
Charlottesville, VA 22908 United States
E-mail: girija@virginia.edu

Kathryn M Ramsey
University of Rhode Island
CBLS 481
120 Flagg Road
Kingston, RI 02881 United States
E-mail: kramsey@uri.edu

Charlene Ranadheera
Public Health Agency of Canada
1015 Arlington Street
Winnipeg, Manitoba R3E 3R2 Canada
E-mail: charlene.ranadheera@canada.ca

Doug Reed
University of Pittsburgh
3501 Fifth Avenue
9043 BST3
Pittsburgh, PA 15261 United States
Tel. 412.648.9290
E-mail: dsreed@pitt.edu

Pavel Rehulka
University of Defence, Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: pavel.rehulka@unob.cz

Helena Rehulkova
University of Defence, Faculty of Health Sciences
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: helena.rehulkova@unob.cz

Patricia Renesto
TIMC-IMAG UMR5525, CNRS-UGA
Institut Jean Roget
Grenoble, 38700 France
E-mail: patricia.renesto@univ-grenoble-alpes.fr

Lydia Roberts
Rocky Mountain Laboratories, NIAID, NIH
Hamilton, MT 59840 United States
E-mail: lydia.roberts@nih.gov

Patrik Ryden
Umeå University
901 87
Umeå, 901 87 Sweden
E-mail: patrik.ryden@umu.se

Shib Shankar Saha
Yamaguchi University
Hirai keneijutaku 309-2,
Yamaguchi, 753-0831 Japan
Tel. +818062417706
E-mail: shib.vet@gmail.com

Emelie Näslund Salomonsson
FOI - Swedish Defence Research Agency
Umeå, Sweden
E-mail: emelie.salomonsson@foi.se

Marina Šantić
Faculty of Medicine, University of Rijeka
22. lipnja 17
Rijeka, HR-51000 Croatia
Tel. +385 51266449
E-mail: marina.santic@medri.uniri.hr

Patrick Sanz
NIAID
5601 Fishers Lane
Rockville, MD 20892 United States
Tel. 240.627.3363
E-mail: sanzp@nih.gov

Anne Grey Savitt
State University New York Stony Brook
Life Sciences Building
Stony Brook, NY 11794-5222 United States
E-mail: anne.savitt@stonybrook.edu

Deanna Schmitt
West Liberty University
208 University Drive
College Union Box 139
West Liberty, WV 26074 United States
Tel. 304.336.8576
E-mail: deanna.schmitt@westliberty.edu

Benji Schwarz
Rocky Mountain Laboratories
Hamilton, MT 59840 United States
E-mail: benjamin.schwarz@nih.gov

Alexander Semikhin
National Research Center of Epidemiology and Microbiology N.F. Gamaleya
Gamaleya Str. 18
Moscow, 123098 Russian Federation
Tel. +7-499-193-30-01
E-mail: asemikhin@yandex.ru

Valeria Sesko (Sheshko)
Faculty of Military Health Sciences, University of Defence
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
Tel. +420973255203
E-mail: valeria.sesko@unob.cz

Claire Siebert
TIMC-IMAG UMR 5525 - UGA CNRS, CHU Grenoble Alpes
Grenoble, France
E-mail: siebert.claire@gmail.com

Anders Sjöstedt
Umeå University
Umeå, 90185 Sweden
E-mail: anders.sjostedt@umu.se

Petra Spidlova
Faculty of Military Health Sciences, University of Defence
Trebesska1575
Hradec Kralove, 500 01 Czech Republic
E-mail: petra.spidlova@unob.cz

Shaun Steele
Washington State University
Pullman, WA 99164 United States
E-mail: shaunsteele@vetmed.wsu.edu

Pavla Stojkova
University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology
Trebesska 1575
Hradec Kralove, 50001 Czech Republic
E-mail: pavla.stojkova@gmail.com

Jiri Stulik
Faculty of Military Health Sciences
Trebesska 1575
Hradec Kralove, 50002 Czech Republic
E-mail: jiri.stulik@unob.cz

David Claes Sundell
Swedish Defence Research Agency (FOI)
Cementvägen 20
Umeå, 90182 Sweden
Tel. 0709723371
E-mail: david.sundell@foi.se

David Thanassi
Stony Brook University
280C Life Sciences Building
Stony Brook, NY 11794-5222 United States
E-mail: david.thanassi@stonybrook.edu

Maka Tsilosani
National Center For Disease Control and Public Health
9 M. Asatiani st., Tbilisi, Georgia
Tbilisi, 0177 Georgia
Tel. 995595385054
E-mail: makatsilosani@gmail.com

Kristen Tummillo
Albany Medical College
Rensselaer, NY United States
E-mail: kristenholland923@gmail.com

Monique L van Hoek
George Mason University
Centreville, VA United States
E-mail: mvanhoek@gmu.edu

Věra Vozandychová
University of Defence
Třebešská 1575
Hradec Králové, 50001 Czech Republic
E-mail: vera.vozandychova@unob.cz

David Wagner
Northern Arizona University
Pathogen and Microbiome Institute
PO Box 4073
Flagstaff, AZ 86001-4730 United States
E-mail: dave.wagner@nau.edu

Anne Grete Wågø
Department of Medical Microbiology
St.Olavs Hospital, Trondheim University Hospital
Trondheim, 7006 Norway
Tel. 004747408016
E-mail: anne.grete.wago@gmail.com

Katherine J. Willett - O'Malley
University of Pittsburgh
3501 Fifth Avenue
9014 BST3
Pittsburgh, PA 15261 United States
E-mail: kjo21@pitt.edu

Mark Williams
OBRRTR/DMID/NIAID
5601 Fishers Lane
Rockville, MD 21704 United States
E-mail: mark.williams4@nih.gov

Hanne Cecilie Winther-Larsen
University of Oslo
Oslo, Norway
E-mail: hannewi@farmasi.uio.no

Matthias Wittwer
Spiez Laboratory
Austrasse 1
Spiez, 3700 Switzerland
E-mail: matthias.wittwer@babs.admin.ch

Lawrence Allen Wolfraim
NIAID/NIH
5601 Fishers Lane
8G46
Rockville, MD 20852 United States
Tel. (240) 627-3393
E-mail: larry.wolfraim@nih.gov

Terry Wu
University of New Mexico Health Sciences Center
1 University of New Mexico
MSC08 4560
Albuquerque, NM 87131-0001 United States
Tel. 505.272.8593
E-mail: TWu@salud.unm.edu

Briana Collette Zellner
University of Toledo
1020 Pine Valley Lane Apt 204
Toledo, OH 43615 United States
Tel. 419.618.4489
E-mail: Briana.Zellner@rockets.utoledo.edu

Xhavit Zogaj
The University of Texas at San Antonio
One UTSA Circle
San Antonio, TX 78249 United States
Tel. 210.458.6141
E-mail: xhavit.zogaj@utsa.edu