ASR 2023

The 32nd Annual Meeting
of the
AMERICAN SOCIETY FOR RICKETTSIOLOGY

JULY 8-11, 2023

SNOWBIRD RESORT
SNOWBIRD, UTAH

AMERICAN SOCIETY for RICKETTSIOLOGY
ASR
Founded 1979

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WELCOME

Dear Attendees of the American Society for Rickettsiology Meeting,

On behalf of the Executive Committee, I am excited to welcome you all to beautiful Snowbird, Utah. The Snowbird Resort was selected due to its tranquil and casual atmosphere, which facilitates conversations and intellectual exchanges among attendees.

As with prior American Society for Rickettsiology (ASR) meetings, we aimed to provide the highest-quality scientific program to attract outstanding researchers in academia, government, and the private sector. We also attempted to promote the diversity, equity, and inclusivity that exists in the Rickettsiology community. We endorsed scientific opportunities for early career investigators to present their work in a collegial yet scientifically rigorous environment. We made available travel awards to trainees and family/childcare support to participants.

For the 32nd ASR meeting, the scientific agenda was divided into eight sessions distributed over 4 days. Half of the 32nd ASR Meeting structure addresses specific discussions for the genera *Rickettsia*, *Coxiella*, *Anaplasma*, *Ehrlichia*, and *Orientia*. The second half of the meeting focuses on a conceptual framework generalizable to most rickettsial bacteria (*e.g.*, epidemiology, diagnostics and vaccine development, host immunity, microbial pathogenesis, cell biology of infection, mutualism, and vector biology). In general, each session will begin with a Keynote Speaker and a Plenary Lecturer. These speakers will provide an overview of the state of the science. Then, we will highlight specific contributions from early career scientists through oral presentations.

The Executive Committee believes that mixing specialties throughout the meeting will incentivize intellectual curiosity and drive conceptual and/or technical innovation. Hence, Keynote Speakers were selected from laboratories that recently contributed to major breakthroughs in the field of rickettsial diseases. Conversely, Plenary Lecturers are guest presenters working in thematic topics that encompass broad conceptual and/or technological appeal; thus, scientifically benefiting our community.

There is no equivalent meeting that focuses exclusively on rickettsial diseases in North America. We anticipate that approximately two hundred attendees representing early career investigators, mid-career scientists, established researchers, and clinicians from academia, government, and private-sector laboratories will attend the 32nd ASR meeting.

I look forward to meeting old friends and making new acquaintances at the 32nd ASR meeting. For almost two decades, I have benefitted from the generosity of the community and enjoyed interacting with members of this vibrant scientific society. It has been a privilege and an honor to serve as the ASR President on your behalf!

Joao H. F. Pedra, PhD
ASR President

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Funding for this conference was made possible (in part) by the grant R13AI176743 from the NIH / National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the U.S. Dept of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

SCIENTIFIC AGENDA

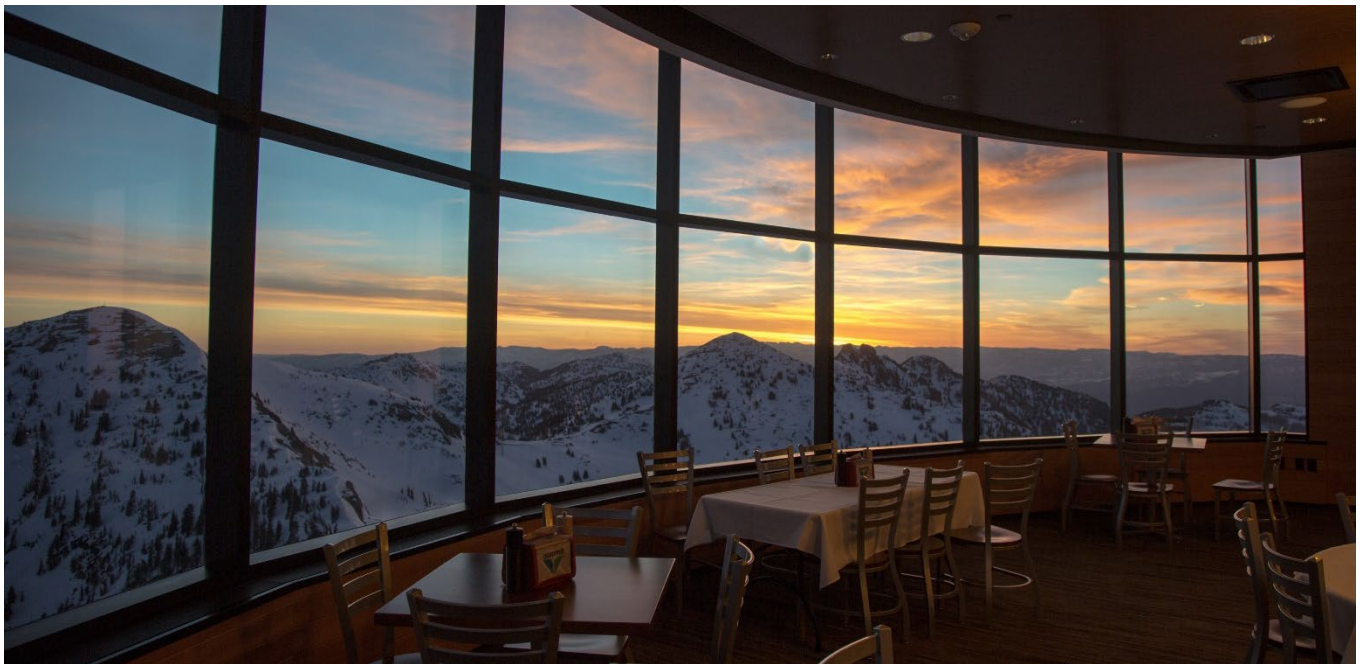
SATURDAY JULY 8

12:00 - 5:30 PM	Registration Desk Open	*Cliff Ballroom Lobby*
12:00 - 3:00 PM	Poster Set Up	*Event Center Tent*
2:00 - 3:00 PM	**EARLY CAREER MENTORING SESSION** *(Pre-Meeting)* Moderator: Stacey Gilk, University of Nebraska Medical Center Panelists: *Michelle Allerdice (CDC) , Lee Fuller (Industry), Shawna Reed (Teaching/Research), Tais Saito (NIH), Kevin Macaluso (Academic Research)*	*Cliff Ballroom I*
3:00 – 3:05 PM	**WELCOME & INTRODUCTIONS** *Joao Pedra, ASR President; Univ. of Maryland SOM*	*Cliff Ballroom I*
3:05 – 4:50 PM	**S1: *ANAPLASMA* AND *EHRLICHIA* - VECTOR TRANSMITTED, VACUOLAR BACTERIAL MANIPULATIONS** Session Chair: Adela Oliva Chavez, Texas A&M Univ.	
3:05 PM	1A. Plenary Overview: Immune system optimization in a variable world - *Ann Thomas Tate, Vanderbilt University*	
3:35 PM	1B. Keynote: New insights into *Anaplasma phagocytophilum* pathogenesis: exploitation of multi-vesicular body biogenesis and sphingolipid signaling - *Jason Carlyon, Virginia Commonwealth University*	
4:05 PM	1C. Mechanism of action of a novel *in vivo* virulence factor of *Ehrlichia* - EHF0962 - *Rory Chien, The Ohio State University*	
4:20 PM	1D. *Anaplasma phagocytophilum AipA* interacts with CD13 to elicit signaling that benefits host cell invasion - *Mary Clark Lind, Virginia Commonwealth University*	
4:35 PM	1E. *Anaplasma phagocytophilum*-infected neutrophils reverse-transmigrate dermal microvascular barriers to disseminate in a 3D model - *Nathaniel Nenortas, Uniformed Services University*	
4:50 – 5:00 PM	BREAK	*Ballroom I Lobby*
5:00 – 5:30 PM	**ASR LECTURE: THE INEQUITY OF INFECTIOUS DISEASE BURDEN AND OUTCOMES IN GLOBAL HEALTH** *Guy Palmer, Washington State University*	*Cliff Ballroom I*
5:30 – 7:00 PM	**WELCOME RECEPTION** *(Dinner on your own at the resort)*	*Golden Cliff*

SUNDAY JULY 9

7:00 - 8:00 AM	Continental Breakfast	*Golden Cliff*
7:00 - 12:00 PM	Registration Desk Open	*Ballroom I Lobby*
8:00 – 9:45 AM	**S2: VECTOR CONTRIBUTION TO RICKETTSIAL PATHOGENESIS** Session Chair: Kathryn Reif, KSU	*Cliff Ballroom I*
8:00 AM	2A. Plenary Lecture: Genome-wide pooled CRISPR screen in arthropod cells - *Norbert Perrimon, HHMI/Harvard University*	
8:30 AM	2B. Keynote Speaker: Acquired tick resistance - *Erol Fikrig, Yale University*	
9:00 AM	2C. Ehrlichial manipulation of its vector, *Ixodes scapularis* *Joseph Aspinwall, National Institutes of Health*	
9:15 AM	2D. Effect of *Anaplasma* spp. transmission on neutrophil migration and collagen structure - *Adela Oliva Chavez, Texas A&M University*	
9:30 AM	2E. Stressed for success: how the unfolded protein response in *Ixodes scapularis* intertwines with pathogen survival - *Kaylee Vosbigian, Washington State University*	
9:45 - 10:15 AM	REFRESHMENT BREAK	*Ballroom I Lobby*
10:15 - 12:00 PM	**S3: TICK-BORNE SPOTTED FEVER GROUP RICKETTSIA** Session Chair: Rebecca Lamason, MIT	*Cliff Ballroom I*
10:15 AM	3A. Plenary Lecture: The molecular basis for *Chlamydia*-mediated remodeling of epithelial cells in the upper genital tract - *Raphael Valdivia, Duke University*	
10:45 AM	3B. Keynote: Manipulation of host cell structures by spotted fever group Rickettsia - *Matthew Welch, UC Berkeley*	
11:15 AM	3C. Host glutathione is required for proper septation, actin-based motility, and virulence of *Rickettsia parkeri* - *Thomas Burke, UC Irvine*	
11:30 AM	3D. Quantitative analysis of morphogenesis and growth dynamics in *Rickettsia parkeri* - *Wanda Figueroa-Cuilan, Johns Hopkins University*	
11:45 AM	3E. New biology from an old antigen: a role for the 17kDa surface lipoprotein in rickettsial cell envelope maintenance - *Brandon Sit, Massachusetts Institute of Technology*	
12:00 – 1:00 PM	**BUSINESS MEETING**	*Cliff Ballroom I*

1:00 – 3:00 PM	**POSTER SESSION I (Odd #s) & LUNCH**	*Event Center Tent*
3:00 – 6:30 PM	*Free Time / Optional Alpine Activities ($)*	*Alpine Area*
6:30 - 9:00 PM	**ASR GROUP DINNER** *(included w/registration fee)*	*The Summit*
5:45	*Meet at the Aerial Tram base for the tram ride to the Summit.*	
	Trams leave promptly at 6:00 / 6:20 / 6:40	



MONDAY JULY 10

7:00 - 8:00 AM	Continental Breakfast	*Golden Cliff*
7:00 - 2:00 PM	Registration Desk Open	*Ballroom I Lobby*
8:00 - 9:45 AM	**S4: HOST IMMUNE RESPONSES AGAINST RICKETTSIAL AGENTS** Session Chair: Sean Riley, University of Maryland	*Cliff Ballroom I*
8:00 AM	4A. Plenary Lecture: Impact of immunity to vector arthropod bites on pathogen transmission - *Jesus Valenzuela, National Institutes of Health*	
8:30 AM	4B. Keynote: Dysregulated Innate and Cellular Immunity During Acute *Orientia tsutsugamushi* Infection - *Lynn Soong, University of Texas Medical Branch*	
9:00 AM	4C. Reversion of *cbu0533* mutation in *Coxiella burnetii* Nine Mile II RSA439, clone 4 results in LPS elongation and increased virulence in a guinea pig model of infection - *Carrie Long, National Institutes of Health*	
9:15 AM	4D. The role of polysaccharide synthesis operon in modulating *Rickettsia*-endothelial cell interactions - *Smruti Mishra, Stony Brook University*	
9:30 AM	4E. Unveiling a new strategy of *Rickettsia*: APRc moonlighting as an evasin by recruiting human complement regulator C4BP - *Isaura Simoes, Biocant, Portugal*	
9:45 – 10:15 AM	REFRESHMENT BREAK	*Ballroom I Lobby*
10:15 - 12:00 PM	**S5: CELL BIOLOGY OF INFECTION** Session Chair: Stacey Gilk, Univ. of Nebraska Medical Center	*Cliff Ballroom I*
10:15 AM	5A: Plenary Lecture: How co-morbidities affect disseminated infection with non-Typhi *Salmonella* - *Renee Tsolis, UC Davis*	
10:45 AM	5B: Keynote: The cell biology and pathogenesis of *Orientia tsutsugamushi* - *Jeanne Salje, University of Cambridge*	
11:15 AM	5C. Bacterial dissemination and protective innate immune responses in an intradermal inoculation mouse model of scrub typhus - *Yuejin Liang, UTMB*	
11:30 AM	5D. Mapping the *Chlamydia trachomatis* conventional type III secreted effector-host interactome - *Brianna Steiert, University of Iowa*	
11:45 AM	5E. The rickettsial effector, *RickA*, contributes to cell-to-cell spread - *Joseph Tran, UC Berkeley*	

12:00 – 2:00 PM **POSTER SESSION 2 (Even #s) & LUNCH** *Event Center Tent*

2:00 – 3:45 PM **S6: EPIDEMIOLOGY, DIAGNOSTICS, AND VACCINE DEVELOPMENT** *Cliff Ballroom I*

Session Chair: Joao Pedra, Univ. of Maryland SOM

2:00 PM 6A: Plenary Lecture: Novel ecological features of emerging Rocky Mountain spotted fever at the US-Mexico border - *Janet Foley, University of California, Davis*

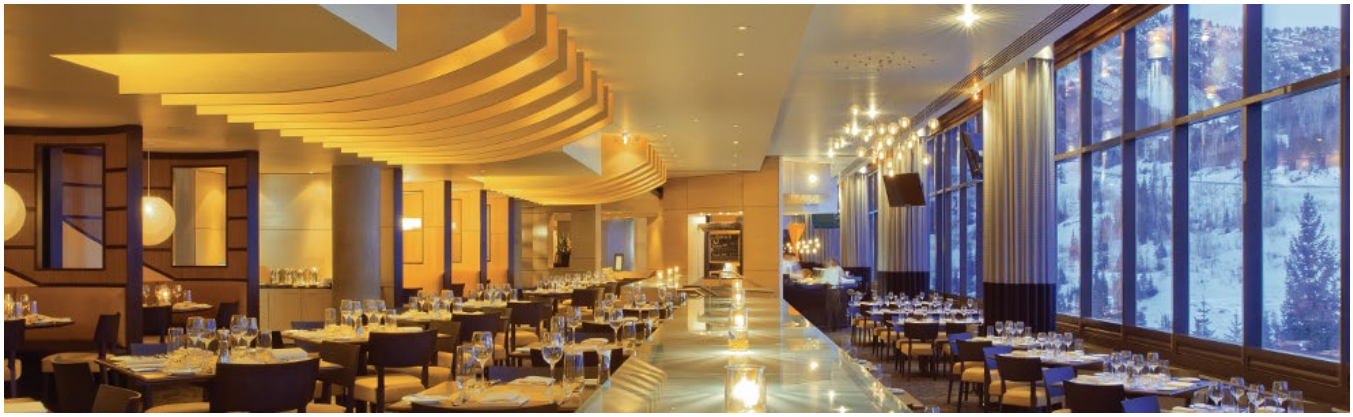
2:30 PM 6B. Keynote: Evolution of Ehrlichia Vaccine Development in the 21st Century - *Jere McBride, University of Texas Medical Branch*

3:00 PM 6C. Modified live *Anaplasma marginale* vaccine developed by targeted mutagenesis stimulates protective immunity against wild type infection from infected tick feeding - *Jonathan Ferm, University of Missouri*

3:15 PM 6D. Serologic and molecular testing for the diagnosis of *Rickettsia*, *Anaplasma*, and *Ehrlichia* infections in individuals in Maryland - *Kristin Mullins, University of Maryland*

3:30 PM 6E. Adjuvanting Coxevac® with QuilA® skews *Coxiella burnetii*-induced inflammatory responses towards a sustained Th1-CD8+-mediated activation and increases protection in an experimental goat model - *Sara Tamaiuolo, University of Ghent, Belgium*

Free evening / Dinner on your own



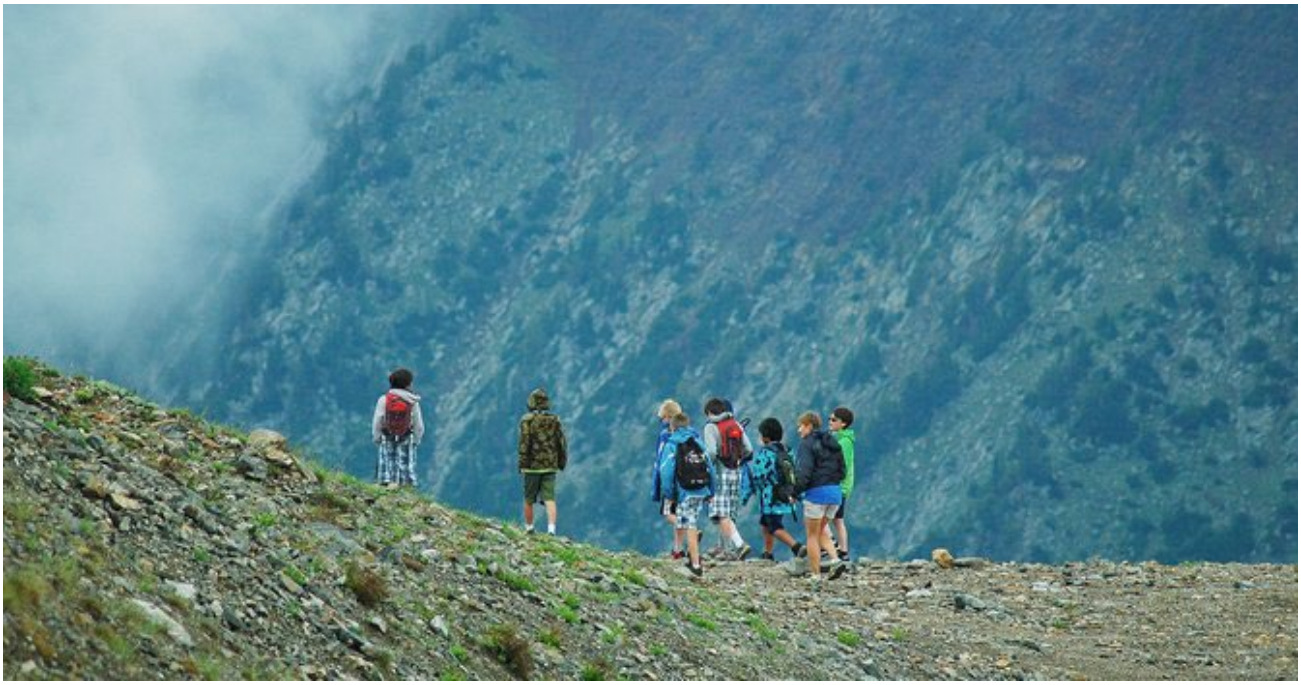
TUESDAY JULY 11

7:00 - 8:00 AM	Continental Breakfast	*Golden Cliff*
7:00 - 12:00 PM	Registration Desk Open	*Ballroom I Lobby*
8:00 - 9:45 AM	**S7: *COXIELLA* BIOLOGY AND PATHOGENESIS** Session Chair: Shawna Reed, Quinnipiac University	*Cliff Ballroom I*
8:00 AM	7A: Plenary Lecture: Conserved strategies to attack the replication niche of intravacuolar pathogens - *Ralph Isberg, Tufts University*	
8:30 AM	7B. Keynote: Establishing the intracellular niche during *Coxiella* infection - *Stacey Gilk, University of Nebraska Medical Center*	
9:00 AM	7C. Host inducible LD-transpeptidase dependent cell envelope remodeling is a major determinant of developmental morphogenesis in *Coxiella burnetii* and *Legionella pneumophila* - *Kelsi Sandoz, Cornell University*	
9:15 AM	7D. Manipulation of TNFα signaling by *Coxiella burnetii* during infection - *Chelsea Osbron, Washington State University*	
9:30 AM	7E. *Coxiella burnetii* modulates host pro-inflammatory and microRNA response - *Rahul Raghavan, University of Texas San Antonio*	
9:45 – 10:15 AM	REFRESHMENT BREAK	*Ballroom I Lobby*
10:15 - 12:00 PM	**S8: VECTOR-MICROBE-HOST INTERACTIONS** Session Chair: Kelsi Sandoz, Cornell University	*Cliff Ballroom I*
10:15 AM	8A: Plenary Lecture: A *Wolbachia* secreted effector disrupts host endocytosis - *Irene Garcia Newton, Indiana University*	
10:45 AM	8B. Keynote: Prospects for a live-attenuated rickettsial vaccine - *Ulrike Munderloh, University of Minnesota*	
11:15 AM	8C. Regulation of the wound healing response at the tick-skin interface - *Luisa Valencia, University of Maryland SOM*	
11:30 AM	8D. Targeted tag mutagenesis employed in studies to define *Ehrlichia* translocated factor-1 (Etf-1) interactions with host cellular proteins - *Dominica Genda, University of Missouri*	
11:45 AM	8E. National MALDI-TOF MS database of *Dermacentor variabilis* and *Dermacentor similis* - *Maria Galletti, CDC*	
12:00 – 12:15 PM	**CLOSING REMARKS BY THE 2024 PRESIDENT**	*Cliff Ballroom I*

CHILDCARE/FAMILY ASSISTANCE GRANTS

The American Society for Rickettsiology strives to make its meeting diverse, equitable, and inclusive. At the 2022 Annual Conference, the exit survey indicated that approximately 25% of the respondents were interested in child and family support services. Of those interested, 10% (mostly women and junior investigators) indicated they were more likely or inclined to come to the next conference should they be provided financial assistance. Therefore, for the 2023 Annual Conference we made seven (7) awards available to attendees for childcare/family responsibilities. Grant money was generously provided through sponsorship and donations.

For more information and future consideration, please contact the Executive Committee of the American Society for Rickettsiology.



TRAVEL AWARDEES

In recognition of excellent research contributions, the following attendees were selected for 2023 Travel Awards:

First name	Last name	University
Het	Adhvaryu	University of Arkansas for Medical Sciences
Olalekan Chris	Akinsulie	Washington State University
Meghan	Bacher	University of California, Berkeley
Maria	Biancaniello	PCOM
Thomas P.	Burke	University of California, Irvine
Jacob	Cassens	University of New Mexico
Rory	Chien	The Ohio State University
Sabrina	Clark	Texas A&M University
Nan	Duan	The Ohio State University
Jonathan	Ferm	University of Missouri System
Wanda M	Figueroa-Cuilan	Johns Hopkins Medical Institute
Dominica	Genda	University of Missouri
Luke	Helminiak	Stony Brook University
Dipak	Kathayat	Cornell University
Yuejin	Liang	University of Texas Medical Branch
Mary Clark	Lind	Virginia Commonwealth University
Paige	McCaslin	University of Iowa
Smruti	Mishra	Stony Brook University
Nathaniel	Nenortas	Uniformed Services University Health Sciences
Adela	Oliva Chavez	Texas A&M University
Chelsea	Osbron	Washington State University
Curtis	Read	Virginia Commonwealth University
Brandon	Sit	Massachusetts Institute of Technology
Maggie	Sladek	University of Nebraska Medical Center
Brianna	Steiert	University of Iowa
Chanakan	Suwanbongkot	University of South Alabama
Ivia	Thiong'o	University of Wyoming, Laramie
Joseph	Tran	University of California, Berkeley
Luisa	Valencia	University of Maryland
Kaylee	Vosbigian	Washington State University

ABSTRACT REVIEWERS

Special thanks to our 2023 reviewers for their diligence in blind reviewing our abstract submissions:

Kelly Brayton	Washington State University
Lisa Brown	Georgia Southern University
Thomas Burke	University of California, Irvine
Jason Carlyon	Virginia Commonwealth University
Elizabeth Di Russo Case	University of Wyoming
Adela Oliva Chavez	Texas A&M University
Liliana Crosby	University of Florida
Alison Fedrow	Shippensburg University
Jere McBride	University of Texas Medical Branch
Irene Newton	Indiana University
Jason Park	Washington State University
Sean Riley	University of Maryland College Park
Isaura Simoes	Biocant
Erin van Schaik	Texas A&M University

INVITED SHORT TALKS

S1-C

Mechanism of Action of a Novel *in vivo* Virulence Factor of *Ehrlichia* — *EHF0962*

Rory Chia-Ching Chien, Mingqun Lin, Yasuko Rikihisa
The Ohio State University, Columbus, USA

Infection of humans and animals with *Ehrlichia* spp., blood-borne obligate intracellular bacteria, potentially causes life-threatening diseases collectively called “Ehrlichiosis”. To overcome the complex mammalian immune system, establish infection by physiological adaptation, and cause diseases within the host, *Ehrlichia* needs to utilize additional strategies (e.g., *in vivo* virulence factors) to those required to infect eukaryotic cells in culture. Our laboratory developed a mouse model of ehrlichiosis using *Ehrlichia japonica* (*Eja*) which causes fatal disease in mice in a dose-dependent manner (LD_{50} = 100 bacteria). We created a library of *Eja* mutants by using Himar1 transposon mutagenesis system that randomly inserts transposon into the *Eja* genome. Using this mutant library, we found that the mutant H59 is the clone that has a Himar1 insertion in *EHF0962*. *EHF0962* encodes a hypothetical protein EHF0962 (13.5 kDa) that is conserved among *Ehrlichia* spp. We verified the production of native EHF0962 protein in wild-type (WT) *Eja* and its absence in H59 due to disruption of *EHF0962* by Himar1 insertion. *EHF0962* is dispensable for infection of cultured mammalian and tick cell lines. Compared to WT *Eja*, H59 IP- or IV-challenged mice showed delayed mortality and had significantly reduced bacterial loads in the blood, liver, and spleen, suggesting that *EHF0962* is a critical *in vivo* virulence factor. Obligate intracellular bacteria have a short, non-proliferating extracellular stage in their developmental cycle to facilitate cell-to-cell spreading and to establish infection. We found that host cell-free H59 rapidly lost infectivity in the mouse plasma with/without heat inactivation, heat-inactivated fetal bovine serum, or DMEM. These findings suggest that *EHF0962* mediates *Ehrlichia* resistance at the extracellular stage to promote infection. We further investigated the biomolecular mechanisms by which *EHF0962* confers resistance to host cell-free *Eja*. This study discovered a unique strategy of *Ehrlichia* spp. to promote *in vivo* infection.

S1-D

Anaplasma phagocytophilum AipA interacts with CD13 to elicit signaling that benefits host cell invasion

Mary Clark Lind[1], Waheeda Naimi[1], Curtis Read[1], Mallika Ghosh[2], Tavis Sparrer[1], Linda Shapiro[2], Jason Carlyon[1]

[1]Virginia Commonwealth University, Richmond, USA. [2]University of Connecticut School of Medicine, Farmington, USA

Human granulocytic anaplasmosis is an emerging tick-borne disease for which no vaccine and limited therapeutic options exist. The causative agent, *Anaplasma phagocytophilum*, is an obligate intracellular bacterium that invades neutrophils using outer membrane-localized invasins that engage specific host cell receptors. AipA is one of three known *A. phagocytophilum* invasins that is critical for infection. A yeast two-hybrid screen identified an interaction between AipA and the C-terminal domain of CD13, a multifunctional protein that upon engagement initiates enzymatic activity, Src and Syk kinase signaling, and endocytosis. Using co-immunoprecipitation, we confirmed the AipA-CD13 interaction and validated that it requires AipA residues 9-21. Inhibition of CD13 enzymatic activity had no effect on bacterial binding to or invasion of host cells. Treating promyelocytic HL-60 cells and neutrophils with an antibody targeting the CD13 C-terminus reduced *A. phagocytophilum* infection by 38%. Conversely, CD13 overexpression on HEK-293T cells increased permissiveness to infection by 40%. Infection of peripheral blood neutrophils in CD13-/- mice was reduced by 40%, indicating that *A. phagocytophilum* utilizes CD13 to invade neutrophils *in vivo*. Because engagement of CD13 is known to elicit Src and Syk signaling, and both kinases have been implicated in *A. phagocytophilum* infection, their relevance to invasion was evaluated. Src and Syk were found to be activated during *A. phagocytophilum* binding and invasion. Specific pharmacologic inhibition of Src reduced infection by 61%, while targeted inhibition of Syk had no effect. Blocking the CD13 C-terminus reduced the ability of *A. phagocytophilum* to activate Src and Syk. Treating HL-60 cells with a CD13-crosslinking antibody that activates Src and Syk restored infectivity of $AipA_{9-21}$ antisera-treated *A. phagocytophilum* organisms. These results demonstrate that *A. phagocytophilum* AipA binds CD13 which induces its crosslinking to elicit a Src signaling pathway that benefits invasion of host cells.

S1-E

Anaplasma phagocytophilum-infected neutrophils reverse-transmigrate dermal microvascular barriers to disseminate in a 3D model

Nathaniel Nenortas, Dennis Grab, Stephen Dumler
USUHS, Bethesda, USA

Anaplasma phagocytophilum (Aph), a non-motile obligate intracellular tick-transmitted bacterium, causes human anaplasmosis. After dermal inoculation, Aph disseminates hematogenously by unknown mechanisms. We hypothesized that neutrophils recruited to the tick bite become infected and reverse transmigrate using a recently discovered process linked to severe inflammatory disease. Alternately, transient endothelial cell infection transfers Aph to tethered neutrophils within the vasculature. We used 3D dermal DAX-1 models with collagen type-I matrix (dermis) and human dermal microvascular endothelial cell (HDMEC) microvessels, and Transwells with HDMECs on the bottom to expose the basolateral (dermal) surface. When cell-free (cf) Aph-mCherry (vs. no cfAph) were added to the dermal side, 2.8-fold ($p<0.05$) more neutrophil HDMEC extravasation occurred; no infected HDMEC were observed. <1% of neutrophils became infected by cfAph in the dermis observed in the process of reverse migration ≥1,050 µm from the HDMECs; infected HDMEC were not observed. To assess neutrophil reverse transmigration, neutrophils ± cell-free Aph were added to the DAX-1 dermal access port, ± HDMEC TNF-stimulation to generate chemotactic gradients. TNF-stimulation increased uninfected neutrophil reverse migration (2.9 ± 0.1-fold, $p<0.005$) but did not affect Aph-infected neutrophils. Dermal compartment supernatants containing Aph-infected neutrophils in DAX-1 models expressed high IL-8, CXCL1, and CCL3 concentrations compared to uninfected neutrophils. Real-time trajectory measurements showed Aph-neutrophil chemokinesis (random motility) rather than chemotaxis, moving ≤ 250 µm into the dermis. In Transwell models that directly examine reverse neutrophil transmigration, Aph-neutrophils transmigrated HDMEC barrier basolateral faces more efficiently than uninfected cells (2.5 ± 0.4-fold, $p<0.05$); no infected HDMEC were observed at any stage. These data support the model that non-motile cfAph alone activate HDMEC recruitment and extravasation of neutrophils that follow a chemotactic gradient in the dermis, where they become infected, alter expression of key neutrophil functions (including chemokine ligand and receptor expression), and reverse transmigrate as the mechanism of dissemination.

S2-C

Ehrlichial Manipulation of its Vector, *Ixodes scapularis*

Joseph Aspinwall, Diane Cockrell, Barbra Weck, Tais B. Saito
NIAID, Hamilton, USA

Tick-borne bacterial pathogens manipulate the behavior and survival of their arthropod vectors to improve bacterial fitness. Some pathogens are known to alter tick motility, survival, reproduction, and tolerance of high temperature conditions. *Ehrlichia muris* subsp. eauclairensis (EME), a tick-borne human pathogen, has been found in the synganglion of its nymphal tick vector. EME is also capable of robust infection of ISE6 cells, a neuron like cell culture from *Ixodes scapularis*. Interestingly, our preliminary observations indicate that EME-carrying *I. scapularis* ticks appear to be more active and survive longer than their uninfected counterpart. The objective of this project is to identify synganglion protein expression changes induced by EME infection and comparatively evaluate changes in tick biology and feeding behavior in ticks carrying EME compared to an uninfected control. To better understand the dynamics of EME infection in the tick synganglion, we first use single cell RNA sequencing to characterize the transcriptional profile of ISE6 cells during EME infection. Our results identify specific clusters associated with the presence of EME. We also perform proteomic analysis of tick synganglia from EME-carrying *I. scapularis* nymphs and uninfected controls over the course of feeding. Finally, we evaluate tick behavior during larval and nymphal feeding in EME-carrying, and uninfected *I. scapularis* ticks. Nymphs infected with EME show an increased attachment rate as compared to the uninfected control, clearly demonstrating behavioral effects of EME during a life phase where EME is known to be present in the synganglion of EME-carrying ticks.

S2-D

Effect of Anaplasma spp. transmission on neutrophil migration and collagen structure

Charluz Arocho Rosario[1], Vidhya Shree Ravi[2], Jacob Underwood[1], Cross Chambers[1], Cristina Harvey[1], Branden Pierce[1], Susan Noh[3,4], Alex Walsh[2], **Adela Oliva Chavez**[1]

[1]Department of Entomology, Texas A&M University, College Station, USA. [2]Department of Biomedical Engineering, Texas A&M University, College Station, USA. [3]Program in Vector-borne Disease, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, USA. [4]USDA-ARS, Animal Diseases Research Unit, Pullman, USA

Anaplasma phagocytophilum and *Anaplasma marginale*, the causative agent of Human Granulocytic Anaplasmosis (HGA) and Bovine Anaplasmosis, respectively, are obligate intracellular bacteria transmitted by ticks. Despite the importance of the skin as the first site of infection, the cellular and molecular events that take place at the bite site during intracellular bacterial transmission and the molecular factors that facilitate bacterial dissemination remain poorly defined. We recently showed that *A. phagocytophilum* transmission results in the activation of neutrophil chemotaxis pathways in the skin. Interestingly, it also led to the downregulation of genes encoding extracellular matrix (ECM) components and upregulation of metalloproteinases, suggesting that A. phagocytophilum affects collagen integrity at the bite site. Similarly, *A. marginale* transmission leads to upregulation of chemotaxis signaling genes, including those involved in neutrophil migration. However, transcriptional regulation of collagen encoding genes in response of *A. marginale* transmission varied between animals. Herein, we describe the effect of intracellular tick-borne bacterial transmission on collagen integrity in the dermis and epidermis of cattle infested with *A. marginale*-infected ticks, uninfected ticks, and normal skin, using second harmonic generation (SHG) microscopy and histology. Similarly, we show the impact of *A. phagocytophilum* transmission in mice skin collagen. Further, we examine the effect of *A. phagocytophilum* transmission on neutrophil numbers at the bite site and its effect on neutrophil metabolism *in vitro*. Understanding the molecular events that take place at the bite site might help us find alternative therapeutics or phrophylatics to stop the transmission and establishment of these pathogens.

S2-E

Stressed for success: How the Unfolded Protein Response in *Ixodes scapularis* intertwines with pathogen survival

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Washington State University, Pullman, USA

In 2019, over 50,000 tickborne disease cases were reported to the CDC. Of these cases, 68% were caused by the Lyme disease spirochete, *Borrelia burgdorferi,* and 15% due to *Anaplasma phagocytophilum*, the causative agent of anaplasmosis. Both pathogens are transmitted by the North American deer tick, *Ixodes scapularis*. Most research on arthropod immunity has been modeled in *Drosophila*. However, immunity is divergent across arthropod species. How *I. scapularis* immunity influences acquisition, maintenance, and transmission of pathogens is incompletely understood. The unfolded protein response (UPR), a cellular stress response, is comprised of three transmembrane receptors, IRE1α, PERK, and ATF6, and has recently been linked to tick immunity. This led to our current question: how does the UPR gene regulatory network respond to infection and influence pathogen dynamics in ticks? Using a UPR reporter plasmid assay, we determined that ATF6 and a transcription factor downstream of PERK, NRF2, were activated by infection. To predict ATF6 and NRF2-regulated genes, we designed a computational transcription factor binding site prediction model of the *I. scapularis* genome. Human and *Drosophila* orthologs of resulting genes were determined to identify putative biochemical and cellular pathways responding to bacteria. Based on this information, genes were chosen to be knockdown in tick cells using RNAi during infection with *A. phagocytophilum*. Our results suggest the ATF6-regulated gene, STOM, and the NRF2-regulated genes NUP214 and PTK2 all support *A. phagocytophilum* growth. These genes were also found to be upregulated in *A. phagocytophilum* infected ticks. Overall, we have generated a novel approach for predicting UPR gene regulatory networks responding to infection in ticks and identified targets that support pathogen colonization.

S3-C

Host glutathione is required for proper septation, actin-based motility, and virulence of *Rickettsia parkeri*.

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Spotted fever group *Rickettsia* are obligate intracellular bacterial pathogens that parasitize nutrients from the host cytosol. Inhibiting nutrient uptake is a possible therapeutic strategy to limit disease; however, the role of nutrient acquisition in pathogenesis remains unclear. Glutathione is an abundant reducing agent in bacteria and eukaryotes that can neutralize reactive oxygen species and can also function as a post-translational modification on cysteines. Here, we investigated the effects of blocking host glutathione synthesis on the spotted fever group pathogen *Rickettsia parkeri*. Infection of Vero cells depleted for glutathione with buthionine sulfoximine (BSO) led to a loss of plaque formation and reduced bacterial burdens. BSO had no observable deleterious effect on host cells nor on *R. parkeri* when treated prior to infection, suggesting that the anti-microbial effects are on the inability of the bacteria to uptake host glutathione. Immunofluorescence microscopy revealed that glutathione is required for proper *R. parkeri* septation, as the prevalence of chained bacteria increased over the course of infection. Chained bacteria had fewer actin-tails and were impeded in their ability to spread from cell to cell. Interestingly, *R. parkeri* abundance was significantly more restricted in BSO-treated macrophages than in BSO-treated Vero cells. We investigated the mechanism of bacterial death in macrophages and found that the loss of viability did not activate the inflammasome nor type I interferon, suggesting that loss of glutathione does not lead to bacteriolysis. Ongoing studies are investigating proteome-wide glutathionylation as a post-translational modification in *Rickettsia*, the mechanism of how glutathione contributes to septation, and whether BSO can be used therapeutically to prevent disease in animals. These studies reveal a novel mechanism for how therapeutically inhibiting the abundance of a host nutrient can reduce bacterial virulence.

S3-D

Quantitative analysis of morphogenesis and growth dynamics in R. parkeri

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The Rickettsiales are obligate intracellular Alphaproteobacteria that include important human pathogens. However, their obligate intracellular lifestyle presents technical challenges that have hindered our progress in understanding essential processes. To address this, we aimed to investigate the fundamental mechanisms of morphogenesis and cell wall metabolism using *R. parkeri* as a model for the Spotted Fever Group (SFG). We first analyzed the cell wall composition of *R. parkeri*, which revealed unique features including high abundance of cell wall monomer tripeptides and a lack of LD crosslinking suggesting specific adaptations in cell wall metabolism to support intracellular growth. We also developed a novel fluorescence microscopy approach to quantify the cell morphology of *R. parkeri* in live host cells. We showed that bacterial morphology is maintained stably during exponential growth; however, the fraction of the population undergoing active constriction decreases over the course of infection. Moreover, we demonstrate, for the first time, that localizing fluorescent fusions, e.g. to the cell division protein ZapA is feasible using this approach. To assess the intracellular growth of *R. parkeri* in a high-throughput and high-resolution manner, we developed an imaging-based growth assay that can measure the growth dynamics of 24 infected cultures at a time. We found the *R. parkeri* doubling time to be ~5 hours. As a proof of concept, we applied our tools to quantitatively determine whether MreB, a bacterial actin homolog, contributes to the growth and morphogenesis of *R. parkeri*. We found that chemical inhibition of MreB with MP265 led to cell rounding and slowed growth, suggesting that MreB is required for the growth and shape maintenance of *R. parkeri*. Finally, the quantitative tools we developed may be applied to characterize the contributions of specific factors and/or environmental conditions to *R. parkeri* intracellular growth and morphology.

S3-E

New biology from an old antigen: a role for the 17kDa surface lipoprotein in rickettsial cell envelope maintenance

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Rickettsial pathogens, which cause an array of mild to life-threatening arthropod-borne vascular disease in humans, encode shared and distinct cell surface protein antigens that provoke mammalian immune responses. Although most major *Rickettsia* antigens have been identified for decades, our knowledge of their contributions to infection and pathogen cell biology is limited. One such factor is the 17kDa surface lipoprotein (also called *hrtA*), which was one of the earliest cloned *Rickettsia* genes. The *hrtA* locus is widely used in rickettsial genotyping and diagnostic studies, but the precise biological function of HrtA is unknown. Phenotypic characterization of an *R. parkeri* transposon mutant strain with an insertion in *hrtA* revealed a marked in vitro infection defect in bacterial growth. We found that bacteria lacking *hrtA* have striking cell shape defects, including a bipolar staining phenotype suggestive of impaired cell division. The predicted structure of HrtA contains a folded C-terminal domain with structural resemblance to bacterial pseudopilus components and OmpA-like domains, hinting that HrtA binds to specific extracellular structures and/or the cell wall. Our data are consistent with a model where HrtA functions similarly to other Gram-negative outer membrane lipoproteins like Pal to stabilize the cell envelope. Improved understanding of rickettsial cell surface antigen biology could potentiate the use of these factors as components in vaccines and diagnostic assays for these emerging pathogens.

S4-B

Dysregulated Innate and Cellular Immunity during Acute *Orientia tsutsugamushi* Infection

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The molecular mechanisms of scrub typhus pathogenesis are poorly defined. Using four murine models (outbred-vs-inbred mice, Karp-vs-Gilliam strains), we compared clinical outcomes and pulmonary cellular immune responses. For Karp infection, gross pathology of severely infected mouse organs resembled features of human scrub typhus; mouse lung NanoString and RNAseq analyses consistently revealed M1/Th1/CD8-skewed, but M2/Th2 repressed, profiles. Multicolor flow cytometric analyses of perfused mouse lungs revealed robust/sustained influx and activation of innate immune cells (monocytes, macrophages, neutrophils, NK cells), followed by those of $CD4^+$ and $CD8^+$ T cells, in Karp-infected C57BL/6 mice, but such responses were greatly attenuated during Gilliam infection. The robust cellular responses in Karp-infected B6 mice were positively correlated with early and high levels of cytokine/chemokine levels in sera. Our studies provided new evidence for differential tissue cellular responses against different *Orientia tsutsugamushi* strains, offering a framework for future investigation of strain-related mechanisms of disease control or pathogenesis.

S4-C

Reversion of cbu0533 mutation in *Coxiella burnetii* Nine Mile II RSA439, clone 4 results in LPS elongation and increased virulence in a guinea pig model of infection

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Coxiella burnetii, the causative agent of human Q fever, undergoes lipopolysaccharide (LPS) phase transition during in vitro passage. This transition is marked by truncation of phase I, smooth LPS culminating in a population of phase II LPS-expressing organisms that produce rough LPS with lipid A, select core sugars but no O-antigen. C. burnetii virulence in animal models of infection is directly related to LPS length. Indeed, the Nine Mile I (NMI) strain that expresses phase I LPS is fully virulent. In contrast, the Nine Mile II, clone 4 (NMII) strain that expresses phase II LPS is considered avirulent. Based on avirulence and a large chromosomal deletion in LPS biosynthesis-related genes, NMII is the only C. burnetii strain exempted from US Division of Select Agents and Toxins (DSAT) regulations.

Here, we demonstrate that viable NMII can be recovered from the spleen of guinea pigs infected with NMII. Recovered bacteria produced semi-rough LPS. Whole genome sequencing revealed that the observed LPS structure corresponded to a spontaneous cbu0533-AA168 mutation, reverting the gene back to wild type. This phenomenon was also observed following passage in the first-generation axenic medium ACCM-1 and was named “LPS elongation”. Enhanced virulence associated with LPS elongation was confirmed in vivo via reinfection of guinea pigs. Together, these studies reveal conditions that promote C. burnetii LPS elongation and virulence potential. The genetic basis of LPS elongation demonstrates that genetic disruption outside of the large deletion thought to be responsible for truncated LPS can affect LPS structure. These findings are significant considering their potential impact on C. burnetii diagnostics, regulation, and laboratory safety. Furthermore, we present an alternative, non-revertible strain for future use.

S4-D

The role of polysaccharide synthesis operon in modulating *Rickettsia*-endothelial cell interactions

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Tick-borne obligate intracellular rickettsial pathogens preferentially infect vascular endothelial cells. As the infection progresses, rickettsiae cause endothelial cell injury and immune cell infiltration. Without antibiotic interventions, infections with pathogenic *Rickettsia* species cause severe vasculitis and multi-organ failure. Thus, endothelial responses to rickettsial infections dictate downstream host immune pathways critical in eliminating *Rickettsia*. Recent studies suggest that pathogenic rickettsial organisms actively manipulate endothelial responses for survival and pathogenesis. With the development of genetic tools for *Rickettsia*, recent investigations provided insights into the underlying molecular mechanisms involved in *Rickettsia*-endothelial cell interactions. Using a *kkaebi* random insertional mutagenesis system, we aim to uncover novel genes involved in *Rickettsia*-endothelial cell interactions. Previously, we identified the biological roles of a *polysaccharide synthesis operon* (*pso*) in the O-antigen biosynthesis, surface protein assembly, rickettsial attachment/invasion to host cells, and spotted fever pathogenesis in the mouse infection model. Nevertheless, additional studies are necessary to determine how *pso* impacts rickettsial surface protein assembly, modulates rickettsial attachment to host cells, and manipulates host immune responses during endothelial cell infections. Herein, we demonstrate that the *pso* mutant exhibits cell-type specific alterations in its abilities to attach, invade, and grow within host cells. Furthermore, our results show that the *pso* mutant fails to suppress immune surveillance systems and induces robust pro-inflammatory responses in endothelial cells. These data corroborate that *Rickettsia* actively suppresses host immune responses to survive and replicate within endothelial cells and delays the recruitment of phagocytic cells to the site of infection. Without active immune modulation, the *pso* variant was avirulent and elicited robust immune protection against wild-type *R. conorii* challenge in mice. Our current studies with the *pso* mutant provide a platform to uncover novel molecular mechanisms involved in rickettsial pathogenesis and host immune modulation.

S4-E

Unveiling a new strategy of *Rickettsia*: APRc moonlighting as an evasin by recruiting human complement regulator C4BP

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Several studies have demonstrated that *Rickettsia* are resistant to serum bactericidal effects and can evade complement-mediated killing. Four rickettsial surface proteins have been identified as important contributors to serum resistance. The rickettsial rOmpB, which specifically interacts with factor H, and the proteins Adr1/Adr2, interacting with vitronectin, illustrate two different mechanisms mediating partial survival of *Rickettsia* in human serum through recruitment of regulators of complement activation. We have also recently shown that the strictly conserved retropepsin-type protease APRc acts as a nonimmune Ig-binding protein that can promote serum resistance, thereby serving as a novel evasin, and demonstrating the existence of an additional resistance mechanism through Ig binding.

Herein, we provide evidence of APRc moonlighting through recruitment of the human complement regulator C4 binding protein (C4BP). C4BP is the primary fluid-phase regulator of the classical (CP) and lectin (LP) pathways, regulating complement activation by inhibiting C3 and C5 convertases assembly, accelerating convertase decay, and acting as a co-factor for factor I, which cleaves and inactivates C4b. Using an enzyme immunoassay, we demonstrated that APRc interferes with the activation of the CP and LP. Identification of C4BP as an APRc target was validated by pull-down assays with normal human serum (NHS). ELISA assays further confirmed APRc-C4BP interaction, and evidence for protein-protein complex formation was observed by native protein agarose gel electrophoresis and size exclusion chromatography. Furthermore, we confirmed C4BP binding at the surface of different *Rickettsia* species by incubation with purified C4BP and NHS, by Western blot analysis and whole-cell ELISA. C4BP recruitment by *Rickettsia* and APRc-C4BP interaction are currently being characterized for their role in enhancing immune evasion. Overall, this study has identified a new role for APRc as an evasin and reveals a new mechanism used by *Rickettsia* to modulate complement activation.

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S5-C

Bacterial dissemination and protective innate immune responses in an intradermal inoculation mouse model of scrub typhus.

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Scrub typhus is an infectious disease caused by the bacteria Orientia tsutsugamushi (*Ot*), which is transmitted by infected chigger mites. Severe infection can lead to organ failure and hemorrhage, resulting in fatality without proper treatment. The mechanism of how the host innate immune system responds to this infection remains elusive. By using an intradermal (i.d.) inoculation mouse model and fluorescent dye-labeled *Ot*, we found that dendritic cells (DCs) and macrophages (MΦ) were the major cell subsets responsible for bacterial uptake in dermis and dissemination into draining lymph nodes (dLN), leading to a systemic infection. This process was dependent on the chemokine receptor CCR7. Interferons (IFNs) are known to play a crucial role in microbial infection defense. Our data showed that the levels of IFNs increased significantly in the skin and lymph nodes after infection, prompting us to hypothesize that IFNs may restrict early bacterial dissemination and control systemic infection. To test this hypothesis, we i.d. infected Ifnar1- or Ifngr-deficient mice and found that the lack of IFN-γ, but not IFN-I signaling, resulted in visible skin eschar lesions, higher bacterial burdens in dLN, exacerbated systemic infection, and animal lethality. Our in vitro study further showed that IFN-γ restricted bacterial growth in DCs and MΦ, suggesting that IFN-γ may inhibit bacterial dissemination in vivo by signaling on bacteria-carrying myeloid cells. Overall, our findings reveal a Trojan horse mechanism of bacterial transmission and highlight the crucial role of IFN-γ signaling in host protection against scrub typhus infection.

S5-D

Mapping the *Chlamydia trachomatis* conventional type III secreted effector – host interactome

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Chlamydia trachomatis (*C.t.*) is the most prevalent bacterial sexually transmitted infection and the leading cause of non-congenital blindness worldwide. Infections are often asymptomatic, leading to severe consequences like pelvic inflammatory disease, ectopic pregnancy, and sterility. From the confines of its inclusion, *C.t.* must engage numerous host organelles and signaling pathways to generate a niche that is permissive for replication. To accomplish these feats, *C.t.* releases an array of proteins into the host cell via a type III secretion system (T3SS). These effectors fall into two classes: conventional effectors and inclusion membrane proteins. *C.t.* is predicted to secrete around 60 conventional T3S effectors, however whether they are actually secreted during infection and how they contribute to bacterial pathogenesis remains largely unknown. Using three distinct secretion assays, we identified 37 proteins that are secreted during chlamydial infection. Using affinity purification-mass spectrometry, we have constructed a *C.t.* effector – host protein interactome, identifying a wide array of novel host targets for conventional secreted effectors. From this screen, we have confirmed the interaction between CteG and CETN2, CT181 and Mcl-1, CT631 and ABCD1, and CT584 and multiple nuclear pore complex proteins. Identifying putative host targets of secreted effector proteins is important for elucidating mechanisms by which *C.t.* interacts with its host and may help identify new therapeutic targets. The interactome we have constructed will be fundamental in this pursuit.

S5-E

The Rickettsial Effector, RickA, Contributes to Cell-to-Cell Spread

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Rickettsia parkeri are capable of causing an arthropod-borne spotted fever disease in humans. During infection, these bacteria invade host cells, escape from the primary vacuole, and undergo two temporally separate modes of actin-based motility, mediated by either the effector RickA or Sca2. RickA-driven motility occurs early after invasion, while Sca2-motility occurs later. Why R. parkeri have evolved to use two effectors for actin-based motility has been a mystery, given that other bacteria that undergo actin-based motility use only one. Although Sca2 has been implicated in mediating cell-to-cell spread, the contributions of RickA to pathogenesis are unclear. We hypothesize that RickA also contributes to cell-to-cell spread during infection. To assess the role of RickA in mediating cell-to-cell spread, we performed infectious focus and plaque assays. We found that *rickA*::Tn mutant bacteria infected fewer cells and formed smaller plaques than wild-type bacteria, indicating that RickA contributes to cell-to-cell spread. To clarify how RickA contributes to spread, we performed live-cell imaging on *sca2*::Tn bacteria, which can only undergo RickA-driven motility. We observed RickA-driven motility continuously propelled bacteria against the plasma membrane to form long protrusions into neighboring cells. Some bacteria-containing protrusions retracted back into the cell, whereas others were engulfed into neighboring cells. This indicates that RickA-driven motility directly contributes to spread. We also characterized the localization of RickA and Sca2 throughout a 48-h time course of infection. We observed low frequencies of bacteria with surface-localized RickA throughout infection, peaking at 15 min post infection, whereas Sca2 was surface-localized on bacteria beginning 5 h and peaking at 24 h post infection. Altogether, our data suggest that both RickA-driven and Sca2-driven motility contribute to cell-to-cell spread and that RickA and Sca2 have distinct temporal patterns of surface localization. These differences may underlie distinct roles for RickA versus Sca2 in pathogenesis.

S6-C

Modified live *Anaplasma marginale* vaccine developed by targeted mutagenesis stimulates protective immunity against wild type infection from infected tick feeding

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Anaplasma marginale is responsible for causing bovine anaplasmosis. It is a globally important tick-borne disease for which vaccines are currently not available in the USA. Recently, we developed a targeted disruption mutation deleting the phage head-tail connector protein (*phtcp*) gene which caused bacterial attenuation and provided protective immunity against I.V. infection (mechanical transmission) and disease progression. We extended investigations of the modified live attenuated *phtcp* mutant vaccine (MLAV) in stimulating immunity against tick transmission of virulent *A. marginale* infection. Pathogen-infected *Dermacentor variabilis* adult male ticks, generated by allowing naïve adults to acquisition feed on infected steers, were used for transmission experiments to MLAV and non-vaccinated steers. All animals were monitored for clinical signs and changes in the blood profiles, cell morphology, IgG response, and infection progression for two months. The bacterial infection was assessed both by blood smear analysis and by qPCR. All non-vaccinated steers receiving tick-transmission infection presented fever, lethargy, and inappetence for several days post infection, while vaccinated animals stayed healthy. A significant drop in the packed cell volume and a rise in the bacteremia were observed in non-vaccinated steers, whereas MLAV steers had no drop in packed cell volume and a seven-fold reduction in bacteremia. Contrary to MLAV animals, blood cell morphology of non-vaccinated steers acquiring tick transmission infection developed acute regenerative anemia and infection with high percentages of reticulocytes, activated lymphocytes, band cells (immature neutrophils), and anisocytosis (variation in red blood cell sizes). Both groups of animals developed IgG response following infection; while a steady rise in the antibody response was observed in non-vaccinated animals, a cyclical response was seen in vaccinated animals. This study demonstrates that the *A. marginale phtcp* gene deleted MLAV also stimulates protective immunity against acute bovine anaplasmosis caused by tick transmitted wild type infection.

S6-D

Serologic and molecular testing for the diagnosis of *Rickettsia, Anaplasma,* and *Ehrlichia* infections in individuals in Maryland.

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Rickettsia, Anaplasma, and *Ehrlichia* are underrecognized pathogens of human importance that require specialized testing for clinical diagnosis. To better understand the prevalence and diagnosis of Rickettisal infections, Anaplasmosis, and Ehrlichiosis in Maryland, a retrospective analysis of all serologic and molecular testing for *Rickettsia, Anaplasma*, and *Ehrlichia* species performed for the University of Maryland Medical System from 2018-2022 was conducted. Eight hospitals within the University of Maryland Medical System (Central Maryland and Maryland's Eastern Shore) diagnose *Rickettsia, Anaplasma*, and *Ehrlichia* infections using serologic and molecular testing. Between 2018-2022, an average of 413 individuals were tested for *Rickettsia, Anaplasma*, and *Ehrlichia* each year. On average 315 serologic tests for Rocky Mountain Spotted Fever (RMSF) group *Rickettsia*, 195 serologic tests for *Ehrlichia chaffeensis* (*Anaplasma* serology is not available), and 165 molecular tests for the detection of *Anaplasma* and *Ehrlichia* species were ordered each year. Clinical indications for testing included tick bite, fever, chills, severe headaches, muscle aches, and rash. Overall, 17% of individuals tested were positive for IgG antibodies to RMSF group *Rickettsia*, however only 1% were positive for IgM antibodies. Further, 20% of individuals tested were positive for IgG antibodies to *Ehrlichia chaffeensis*, while <1% were positive for IgM. Acute and convalescent samples were not tested for any individuals. Molecular evidence of infection was found in 8% of individuals tested. Nineteen percent were positive for *Anaplasma phagocytophilum* and the remaining 81% were positive for *Ehrlichia chaffeensis*. Interestingly, only two individuals had positive results from both molecular and serologic testing. Results from serologic and molecular testing show that *Rickettsia, Anaplasma*, and *Ehrlichia* are important human pathogens with the potential to cause significant morbidity. However, lack of follow-up serology and laboratory tests that confirm acute infection make definitive diagnosis difficult. Increased awareness of Rickettsial infections, *Anaplasma,* and *Ehrlichia*, in conjunction with appropriate diagnostics, is needed.

S6-E

Adjuvanting Coxevac® with QuilA® skews Coxiella burnetii-induced inflammatory responses towards a sustained Th1-CD8+-mediated activation and increases protection in an experimental goat model

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Q Fever is a zoonotic bacterial infection due to Coxiella burnetii, leading to reproductive disorders in ruminants. Coxevac® is the EMA-approved veterinary vaccine for protection of cattle and goats against Q Fever. It is the sole solution to control disease diffusion and transmission to humans. Since Coxevac® reduces bacterial shedding and clinical symptoms but does not prevent infection under high infection pressures, novel vaccine formulations are needed to overcome this problem. In this study, a goat vaccination-challenge model was used to evaluate the impact of the saponin-based QuilA adjuvant on Coxevac® immunity. Compared to Coxevac® (Cox) only, vaccination including QuilA (Quil-A Cox) resulted in a transient increase in the mean body temperature (39.9°C vs 38.5°C) and a protracted total serum IgG production. None of the vaccine formulations affected the frequency of peripheral CD4+ and CD8+ T-lymphocytes, though each triggered a different in vitro recall IFNg T-cell response. While Quil-A Cox activated antigen-specific IFNγ production two weeks after both prime and boost dose, Cox only triggered IFNγ production two weeks after the boost. Upon challenge, the Quil-A Cox group showed a stronger systemic immune response reflected in a higher magnitude of total IgG, an increase in circulating CD8+ T-cells and a biphasic antigen-specific IFNγ release compared to the Cox group. In spleen, higher levels of CD8+, NRC1 and IFNγ transcripts were detected in the Quil-A Cox group as opposed to the challenged control group. The latter showed highly expressed pro-inflammatory cytokines, such as IL1B and IL17A in spleens and IL6 in respiratory lymph nodes. In addition, QuilA adjuvant induced a moderate but increased protection efficacy. Overall, the QuilA adjuvant enhanced adaptive immune responses triggered by Coxevac® in C. burnetii-challenged goats, resulting in a stronger total IgG production and a Th1-type CD8+ cell mediated response.

S7-C

Host inducible LD-transpeptidase dependent cell envelope remodeling is a major determinant of developmental morphogenesis in Coxiella burnetii and Legionella pneumophila

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Coxiella burnetii and Legionella pneumophila are two phylogenetically related bacterial pathogens that exhibit extreme intrinsic resistance when they enter a dormant-like state. This enables both pathogens to survive long periods in nutrient-limited environments and transmit disease to susceptible hosts. This process is dependent upon their ability to undergo developmental morphogenesis into phenotypically distinct variants. We currently lack an understanding of the mechanisms that regulate developmental morphogenesis and environmental survival. Here, we show that significant structural changes occur in the cell envelope of C. burnetii and L. pneumophila upon phenotypic differentiation. This coincides with the upregulation of numerous structurally diverse LD-transpeptidases (Ldts) by the general stress response sigma factor, RpoS. In support, LC-MS/MS analysis of peptidoglycan (PG) revealed a significant enrichment in Ldt-catalyzed cross-linking in PG and activation of a new mechanism of cell envelope stabilization called beta-barrel outer membrane (OM) tethering. Ldts are differentially expressed during intracellular models of growth, which profoundly impacts the structure of the cell envelope. Collectively, these findings show that Ldt-mediated remodeling of the cell envelope is a major determinant of developmental morphogenesis in C. burnetii and L. pneumophila. Understanding this mechanism will inform new therapeutic approaches for treating chronic infections caused by these pathogens, as well enable new methods to decontaminate environmental reservoirs during outbreaks.

S7-D

Manipulation of TNFα signaling by *Coxiella burnetii* during infection

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Coxiella burnetii is an obligate intracellular bacteria and the causative agent of the global zoonotic disease Q Fever. Treatment options for *C. burnetii* infection are limited, and the development of new, targeted therapeutic requires a greater understanding of how *C. burnetii* interacts with host immune signaling pathways. In this research, we investigated how *C. burnetii* manipulates TNFα-mediated apoptosis, necroptosis, and NFκB activation to promote bacterial infection. We measured caspase, RIPK, and MLKL activation in *C. burnetii*-infected TNFα/CHX-treated THP-1 macrophage-like cells and TNFα/ZVAD-treated L929 cells to assess apoptotic and necroptotic signaling. Additionally, we measured *C. burnetii* replication over 12 days in RIPK1-kinase-dead, RIPK3-kinase-dead, or RIPK3-kinase-dead caspase-$8^{-/-}$ BMDMs, as these proteins are downstream of TNFα signaling. We found that caspase-8 activation is inhibited during infection, coinciding with increased levels of cFLIP. Furthermore, *C. burnetii* replication was increased in BMDMs lacking RIPK1 kinase activity or caspase-8, but not in BMDMs lacking RIPK3 kinase activity, indicating the significance of caspase-8 and RIPK1 activity related to apoptosis and cytokine signaling to controlling infection. Next, we will interrogate the importance of cFLIP in mediating bacterial inhibition of caspase-8 and analyze the mechanisms by which caspase-8 and RIPK1 regulate cytokine signaling during *C. burnetii* infection. Our results will aid in uncovering the relationships between TNFα signaling components and *C. burnetii* infection, thereby providing necessary information for effectively targeting host innate immune pathways to enhance the immune response against *C. burnetii*.

S7-E

Coxiella burnetii modulates host pro-inflammatory and microRNA response

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Coxiella burnetii, the causative agent of Q fever, typically infects human alveolar macrophages. To elucidate host response, we conducted gene-expression profiling of primary human alveolar macrophages (hAMs) infected with one of four C. burnetii isolates: Nine Mile phase I RSA493, Nine Mile phase II RSA439, Dugway, or Graves. Irrespective of the infecting isolate, C. burnetii activated the expression of pro-inflammatory signaling pathway genes such as those downstream of IL-17 signaling, including genes for numerous cytokines and chemokines. We confirmed this observation by measuring cytokines and chemokines secreted by NMII-infected THP-1 macrophages at several time-points during infection. To further understand the inflammatory response, we conducted a single-cell-RNA-seq analysis of NMII-infected THP-1 macrophages and discovered that infection leads to a range of inflammatory states among macrophages, with some subpopulations seemly more hospitable to C. burnetii than others. In addition to revealing host inflammatory response, our analysis identified several miRNAs that were down- or up-regulated in human macrophages during infection. A detailed exploration of the functions of miR-143-3p, a miRNA whose expression was down-regulated in macrophages during C. burnetii infection, showed that increasing the abundance of this miRNA in human cells results in increased apoptosis and reduced autophagy – conditions that are unfavorable to C. burnetii intracellular growth. In sum, C. burnetii infection elicits a robust pro-inflammatory and miRNA response in human macrophages and the pathogen in turn modulates the host response to promote infection.

S8-B

Prospects for a live-attenuated rickettsial vaccine

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The incidence of diseases caused by tick-borne pathogens in North America has been increasing over the past decades. The majority of illnesses is caused by bacteria, and treatable using antibiotics, although the number of effective drugs is limited. Moreover, outcome may be poor if therapy is delayed, which is not uncommon due to the non-specific nature of disease signs and symptoms. Immunoprevention of these diseases would offer many advantages, in particular for populations residing in areas with limited access to urgent health care. Previous efforts to devise vaccines against tick-borne rickettsiae have had mixed results, causing significant side effects, including vaccine-induced rickettsiosis in the case of a live-attenuated epidemic typhus vaccine used during World War II. Nevertheless, live-attenuated vaccines are among the most effective vaccines available. Early versions of such vaccines lacked a way to select desirable mutants while removing wild-type bacteria, and were unsafe. To overcome these drawbacks, we created a bank of transposon mutants from mildly pathogenic Rickettsia parkeri that cross-protects against spotted fever rickettsioses. Most mutants were cleared within days from infected mouse tissues, and one mutant induced protection against homologous and heterologous challenge. To expand the utility of such mutants, we designed transposons encoding exposed epitopes of the tick-borne rickettsial pathogen Anaplasma phagocytophilum. Epitopes from VirB, Asp55 and Asp62 encoded on a shuttle vector transfected into R. parkeri were transcribed and expressed by R. parkeri mutants, and induced antibody responses in infected mice. Furthermore, we replaced the transposon in R. parkeri mutants with a cassette encoding the same epitopes. As shown for plasmid transformants, such mutants transcribed and expressed the A. phagocytophilum epitopes. This vaccine approach utilizes safe R. parkeri mutants enhanced with immunogenic epitopes from other rickettsial pathogens that can be replaced by other sequences to create a flexible vaccine platform against multiple tick-borne pathogens.

S8-C

Regulation of the wound healing response at the tick-skin interface

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Hard ticks are important hematophagous arthropods of public health relevance due to their role in the transmission of infectious agents. The complex life cycle of hard ticks requires prolonged feeding via attachment to the skin. During feeding, the tick hypostome disrupts the host homeostasis and leads to injury of the skin epidermal and dermal compartments. To ensure successful feeding, ticks counter the host response with bioactive salivary molecules that antagonize host inflammation, coagulation, and nociception. Here, we employ single cell RNA sequencing and spatial transcriptomics to further understand the tick-skin interface and the gene signatures associated with feeding on a mammalian host. We highlight the inflammatory wound healing gene signature elicited by the tick at the host skin, with a particular emphasis on the role of TGF-β signaling in engaging the host wound healing response. Furthermore, we utilized animal models and flow cytometry to validate the TGF-β signaling signature in keratinocytes. Collectively, our work reveals greater insight into mechanisms of tick-mediated regulation of the host wound healing response and broadens our understanding of vector-host interactions.

S8-D

Targeted tag mutagenesis employed in studies to define Ehrlichia translocated factor-1 (Etf-1) interactions with host cellular proteins.

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Ehrlichia chaffeensis is a tick-transmitted bacterium that causes human monocytic ehrlichiosis. Bacterial type four secretion systems (T4SS) are used to translocate effector proteins into host cells. Ehrlichia translocated factor-1 (Etf-1) is the first known T4SS effector. Due to a lack of established genetic manipulation methods, prior research relied on the ectopic expression method to investigate effector functions. With our recently developed targeted mutagenesis methods, we created a mutation introducing an in-frame coding sequence of enhanced green fluorescent protein (EGFP) at the 3' end. The mutation also introduced the mCherry sequence expressed by the Ehrlichia transcriptional elongation factor (tuf) gene promoter. The mutant is expected to constitutively express mCherry as part of bacterial growth, while EGFP expression is linked to Etf1 expression (Etf1-EGFP) from its native promoter and as per bacterial transcriptional regulation. After confirming the mutant's clonal purity, Etf1-EGFP and mCherry expressions were assessed for several days after infecting canine macrophage cells by confocal microscopy. Further, Etf1-EGFP localization within Ehrlichia and host cell fractions and its interacting proteins were evaluated by cellular fractionation, Western blot analysis, co-immunoprecipitation, and proteomic analysis. We observed time-dependent changes in the Etf1 expression and translocation, with predominant expression observed around day five. We detected Etf1 in Ehrlichia-containing phagosomes, host cell cytoplasm, and mitochondria. Proteomic analysis of co-immunoprecipitated proteins from infected macrophages at eight days post-infection identified many host proteins interacting with Etf1, which included 36 from mitochondrial fraction, 66 from cytosol fraction, and 314 proteins isolated from whole cell lysate. In addition, 11 interacting proteins originating from E. chaffeensis were detected. The exact nature of Etf1 interactions with host cellular machinery remains to be defined in this physiologically pertinent system. This is the first study describing targeted mutagenesis in E. chaffeensis aiding investigations of a T4SS effector protein from the bacterial native promoter.

S8-E

National MALDI-TOF MS database of *Dermacentor variabilis* and *Dermacentor similis*

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Ticks have been identified historically by predominantly morphological methods which can involve highly specialized expertise, and more recently, by DNA-based molecular techniques that often incur considerable costs per specimen. Although both methods have been perfected over time, some limitations remain, including challenges with morphological identification of cryptic species, immature stages of some species, and damaged specimens, while specificity and cost still restrain the use of gene-based methods. These limitations are acutely apparent when evaluating large sample sets of regional or national tick surveillance programs. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) was evaluated as a cost-efficient method for tick identification and its utility as a technique to rapidly and accurately resolve morphologically and genetically cryptic species. Over 300 specimens of *Dermacentor variabilis* and *Dermacentor similis*, collected from 19 eastern, central, and western U.S. states were evaluated using an integrative taxonomic approach incorporating classical morphological evaluation, molecular techniques, and MALDI-TOF MS. Resulting datasets were integrated into a broader CDC platform known as MicrobeNet, a free on-line tool and information resource that incorporates collective data for thousands of bacterial pathogens, including a searchable database of MALDI-TOF profiles used for pathogen identification. This publicly available database of tick MALDI-TOF profiles detailing the taxonomy, ecology, geographical distribution, genetics, and pathogens associated with each tick species, represents the first integration of information for medically relevant arthropods into this internationally accessed platform. Future additions of MALDI-TOF MS and metadata associated with other medically relevant tick species into MicrobeNet will provide accurate, accessible, and timely information to public health and scientific communities. Collectively, this work can strengthen vector identification and facilitate national tick surveillance programs in the United States. Efforts are actively ongoing to incorporate similar data for additional hard tick species in the US, as well as potentially cryptic species of medical and veterinary importance.

POSTERS

P01

Ehrlichia chaffeensis TRP120 utilizes short linear motif mimicry (SLiM-icry) to induce a chemokine response in human monocytes

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As an obligately intracellular, gram-negative bacterium and the causative agent of human monocytic ehrlichiosis (HME), *Ehrlichia chaffeensis* has evolved novel survival strategies against host innate immune responses. *E. chaffeensis* exhibits tropism for mononuclear phagocytes and evades immune defenses by secreting effector proteins that manipulate host cell signaling. TRP120 (120-kDA tandem repeat protein) is a crucial effector protein that drives the survival of *E. chaffeensis* as it presents moonlighting functions as a nucleomodulin, ubiquitin ligase, and ligand mimetic. TRP120 is an intrinsically disordered protein containing short linear motifs (SLiMs) that arose *in nihilo* and contribute to its moonlighting effector functions. Published data from our laboratory demonstrate that TRP120 utilizes short linear motif mimicry (SLiM-icry) to activate Notch, Wnt and Hedgehog signaling pathways to promote disease pathogenesis. Interestingly, data from our laboratory also demonstrates that *E. chaffeensis* TRP120 induces chemokine secretion, likely through the utilization of SLiM-icry. To identify the molecular basis of TRP120 chemokine induction during *E. chaffeensis* infection, THP-1 cells were infected with cell-free *E. chaffeensis*, recombinant TRP120-FL (full length), rTRP120-TR (tandem repeat), rTRP120-NTD (N-terminal domain), or rThioredoxin (negative control) and harvested after 48 hours. Enzyme-linked immunosorbent assay (ELISA) revealed that MCP-1(CCL2) is secreted in response to *E. chaffeensis*, rTRP120-FL and rTRP120-NTD, suggesting that there is a SLiM responsible for chemokine induction that is localized to the 50 amino acid N-terminal domain of TRP120. Moreover, our studies reveal that *E. chaffeensis*, rTRP120-FL and rTRP120-NTD upregulate additional chemokines such as CCL20(MIP3a), CXCL8(IL-8), CXCL9 and CXCL10(IP-10) at the transcript and protein level. These findings suggest that while subverting innate immune detection, *E. chaffeensis* likely evolved a mechanism to fine-tune the induction of chemokines to promote monocyte infection.

P02

A novel regulator of Golgi fragmentation, ceramide-1-phosphate, is essential for *Anaplasma phagocytophilum* pathogenesis

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Anaplasma phagocytophilum lives in a modified multivesicular body that receives membrane traffic from numerous organelles including the Golgi apparatus. *A. phagocytophilum* induces Golgi fragmentation to maximize delivery of trans-Golgi derived sphingolipid-rich vesicles into its vacuole, which is essential for conversion to its infectious form. The host cell pathway that *A. phagocytophilum* exploits for this pathogenic mechanism is undefined. Here, we identify the bioactive sphingolipid, ceramide-1-phosphate (C1P), as a novel regulator of Golgi fragmentation. *A. phagocytophilum* infected host cells exhibit increased levels of C1P. Pharmacologic inhibition or siRNA-mediated depletion of ceramide kinase (CERK), the enzyme that produces C1P, blocks *A. phagocytophilum*-induced Golgi fragmentation and bacterial development. Additionally, *A. phagocytophilum* is unable to establish a productive infection in CERK-/- mice. Conversely, downregulation of ceramide-1-phosphate transfer protein (CPTP), which spatiotemporally regulates C1P in the host cell, induces Golgi fragmentation to enhance bacterial development. Furthermore, C1P activates a PKCa/cdc42/JNK signaling axis that results in the phosphorylation of Golgi reassembly stacking proteins (GRASP) 55 and GRASP65 and, consequently, Golgi destabilization. Host cells expressing phosphorylation-resistant GRASP55 and GRASP65 mutants are resistant to *A. phagocytophilum*-induced Golgi fragmentation. These data reveal a novel C1P-regulated PKCa/cdc42/JNK/GRASP signaling pathway that induces Golgi fragmentation and is coopted by *A. phagocytophilum* to complete its infection cycle.

P03

Anaplasma phagocytophilum modulates intracellular calcium levels to facilitate regulated exocytosis of the pathogen-containing vacuole.

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The Gram-negative obligate intracellular bacterium, *Anaplasma phagocytophilum*, is an emergent tick-borne pathogen that causes the potentially fatal disease, human granulocytic anaplasmosis. *A. phagocytophilum* preferentially infects human neutrophils and upon invasion proceeds through a biphasic developmental cycle consisting of both infectious (dense-cored: DC) and non-infectious (reticulate cell: RC) forms. *A. phagocytophilum* growth and development occur within a host-derived vacuole that shares many characteristics of the host multivesicular body (MVB). Unlike MVBs destined for lysosomal fusion, the *A. phagocytophilum* vacuole (ApV) is CD63 negative. Moreover, late-stage ApVs traverse to and fuse with the plasma membrane to release infectious progeny for dissemination to naive cells via a process involving Rab27a and Munc13-4, two proteins required for regulated exocytosis. Here, we investigated the potential involvement of regulated exocytosis in *A. phagocytophilum* infection by measuring host intracellular calcium levels, a known regulated exocytosis trigger, across the *A. phagocytophilum* developmental cycle using the live-cell fluorescent sensor, Fluo-8 AM. Our studies revealed that calcium is modulated at key points during the developmental cycle, with increases occurring at both bacterial entry and upon release from the host. Treating *A. phagocytophilum* infected host cells with chloramphenicol at 16 h post infection (prior to RC-to-DC conversion) inhibited calcium influx into the cytosol and prevented *A. phagocytophilum* release into the extracellular milieu. These data further implicate regulated exocytosis as the mechanism by which *A. phagocytophilum* disseminates from host cells and suggest this process is a bacterial-mediated event.

P04

Inhibition of Ehrlichia Infection by Intracellular Nanobodies that Block Etf-2 Binding to Active RAB5 and Localization to Endosomes

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Ehrlichia chaffeensis, an obligatory intracellular bacterium, infects monocytes/macrophages by sequestering a regulator of endosomal traffic, the small GTPase RAB5 on its membrane-bound inclusions to avoid routing to host-cell phagolysosomes. Ehrlichia translocated factor-2 (Etf-2), an effector of the *Ehrlichia* type IV secretion system is secreted into the host-cell cytoplasm and its C-terminus (Etf-2C) directly binds to GTP-bound RAB5 at high affinity, localizes to ehrlichial inclusions, and interferes with RAB5 GTPase activating protein RABGAP5 from localizing to ehrlichial inclusions. Of 10 distinct anti-Etf-2C nanobodies (Nbs), NbA44 and NbA123 blocked Etf-2C-GFP binding to endogenous RAB5 in co-transfected human cells. mCherry-NbA44 and NbA123 blocked Etf-2-GFP trafficking to E. chaffeensis inclusion surface as well as early endosomes marked with HA-RAB5CA. mCherry-NbA44 and NbA123 also blocked Etf-2 trafficking to endosomes containing EtpE-C-coated latex beads and abrogated inhibition of the endosome maturation mediated by Etf-2. Etf-2, although lacking a RAB GTPase-activating protein (GAP) Tre2-Bub2-Cdc16 (TBC) domain, it contains two conserved TBC domain motifs, namely an Arg finger and a Gln finger, and both Arg188 and Gln245 in Etf-2C are required for Etf-2 localization to early endosomes. The epitopes of NbA44 and NbA123 are within the dual finger motif, but not overlapping, as site-directed mutagenesis revealed that binding to Etf-2 requires only Gln245, and both Arg188 and Gln245, respectively. NbA44 and NbA123 significantly inhibited *E. chaffeensis* infection and the combination of the two Nbs showed synergistic inhibition of near 90%. Our results not only affirm the critical role and mechanisms of Etf-2 functions in *E. chaffeensis* infection, but also demonstrate the utility of nanobodies as intracellular probes, inhibitors, and drugs to decipher biomolecular mechanisms of *Ehrlichia* infection.

P05

Ehrlichia ubiquitination and degradation of mRNA splicing factor hnRNPA2B1

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Obligately intracellular bacterium *Ehrlichia chaffeensis* is the etiologic agent of human monocytotropic ehrlichiosis (HME), a life-threatening, group 1 NIAID emerging zoonotic disease. *E. chaffeensis* selectively infects and replicates in mononuclear phagocytes by secreting multiple bacterial effectors, including the tandem repeat protein 120kDa (TRP120). During infection, TRP120 acts as a nucleomodulin with the ability to bind host cell DNA and have important functions in the nucleus. Previous studies by our laboratory have defined the functional HECT-type E3 ubiquitin ligase activity by which TRP120 mediates K48-ubiquitination and proteasomal degradation of various host target proteins, including nuclear tumor suppressor F-BOX and WD repeat domain-containing 7 (FBW7) and polycomb group ring finger proteins (PCGFs) to promote infection. Using yeast two-hybrid assay, we previously identified the mRNA processing regulator, heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), as a binding partner of TRP120. Recent antiviral investigation has determined a key role of hnRNPA2B1 in modulating innate immune response against infection. These observations led us to hypothesize that TRP120 targets nuclear hnRNPA2B1 for degradation to modulate innate immune responses, thereby promoting infection. We examined the role of hnRNPA2B1 during infection using siRNA knockdown and ectopic overexpression approaches and observed that siRNA hnRNPA2B1 knockdown promoted infection, whereas hnRNPA2B1 overexpression caused an inverse effect. During infection, hnRNPA2B1 nuclear levels were significantly degraded, while ubiquitinated hnRNPA2B1 and total ubiquitinated protein levels were increased. Colocalization and co-immunoprecipitation demonstrated that hnRNPA2B1 interacted with TRP120 during infection. Pulldown assays further determined the tandem repeat domain of TR120 and the prion-like domain of hnRNPA2B1 were required for TRP120-hnRNPA2B1 interaction. Collectively, this study demonstrates that hnRNPA2B1 is degraded during infection by TRP120 E3 ubiquitin ligase activity. Further, this study illuminates the importance of bacterial ubiquitin ligases and the ubiquitin-proteasome system for the infection of *E. chaffeensis*, thereby creating a model for investigation in other bacterial pathogens.

P06

Ehrlichia Co-opts Host Cell NFAT Signaling Using Ligand Mimicry

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The nuclear factor of activated T cells (NFAT) protein family plays a critical role in regulating the immune response. The five NFAT family members (NFAT1-5) are transcription factors canonically activated by calcium signaling (NFAT1-4) and osmotic stress (NFAT5). The FAT family has been implicated in the activation and development of immune cells, as well as cellular processes including proliferation and survival. Given its relation to immune signaling, the NFAT pathway is targeted by pathogens including *Ehrlichia chaffeensis*. It has been previously established that NFAT2 is activated during *E. chaffeensis* infection, and knockdown of NFAT2 negatively influences infection. Recently, four of the five NFAT family members were demonstrated to be upregulated by *E. chaffeensis*, including the differentially activated NFAT5. Furthermore, treatment with a peptide derived from the tandem repeat protein 120 (TRP120) effector protein of *E. chaffeensis* was proven sufficient to activate NFAT2 in THP-1 cells. *E. chaffeensis* is well-known to mimic human ligands with short linear motifs in effector proteins, co-opting host cell signaling to inhibit host cell apoptosis. Thus, it is possible that broad alteration of NFAT signaling by *E. chaffeensis* occurs by a similar mechanism and serves a similar purpose. This study evaluated the capacity of *E. chaffeensis* effector proteins to influence NFAT signaling, and aimed to identify the precise mechanism by which NFAT signaling is manipulated by *E. chaffeensis*.

P07

A Novel T4SS Effector Facilitates *Anaplasma phagocytophilum* Infection by Engaging ER-Golgi Pathway

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Human granulocytic anaplasmosis (HGA) is an emerging tick-borne infectious disease that causes a potentially fatal, severe influenza-like illness. The causative agent of HGA, *Anaplasma phagocytophilum* (*Aph*), is an obligatory intracellular bacterium that proliferates in the membrane-bound compartment (inclusions) in the cytoplasm of neutrophils. Mechanisms of how *Aph* infects, survives, and proliferates in these cells remain largely unknown. *Aph* has the Type IV secretion system (T4SS) that directly inoculates bacterial molecules into human cells to manipulate host cells for its survival and proliferation. Based on bioinformatics analysis and bacterial two-hybrid assay, a hypothetical protein APH0874 was identified as a potential T4SS effector. To experimentally validate APH0874 as a bona fide T4SS effector, *Aph* mutants expressing FLAG-tagged C-terminal APH0874 with/without its C-terminal secretion signal (FLAG-APH0874C or FLAG-APH0874CΔS) were constructed by Himar1 transposon mutagenesis system. Immunofluorescence labeling showed that both native APH0874 and FLAG-APH0874C were secreted and localized on the surface of *Aph*-containing inclusions, whereas FLAG-APH0874CΔS was retained within *Aph*. APH0874 knockdown by *APH0874* anti-sense peptide nucleic acid transfection of *Aph* significantly reduced infection. To investigate the mechanisms and functions of APH0874, GFP-tagged full-length- or different fragments of APH0874 were constructed and transfected into uninfected or *Aph*-infected RF/6A cells. Immunofluorescence labeling showed that APH0874 full-length and APH0874C co-localized with Golgi marker, whereas the N-terminal fragment of APH0874 (APH874N) colocalized with ER marker in uninfected RF/6A cells. In *Aph*-infected cells, full-length and APH0874C encircled *Aph* inclusions, but not APH0874N. These results support APH0874 is indeed secreted by *Aph* into the cytoplasm of host cells in the secretion signal-dependent manner and plays a crucial role in *Aph* infection and inclusion biogenesis by engaging ER and Golgi pathway. Further studies on analyzing APH0874 functional domains and their interacting host proteins will help uncover novel molecular mechanisms of HGA pathogenesis.

P08

Nanobodies Against Type IV Secretion Effector Etf-3 Inhibit *Ehrlichia* Infection and Etf-3-Induced Ferritinophagy

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Ehrlichia chaffeensis is an obligatory intracellular bacterium that infects monocytes and macrophages, and causes human monocytic ehrlichiosis. *Ehrlichia* translocated factor-3 (Etf-3) is secreted into the host cell cytoplasm by bacterial type IV secretion system. Secreted Etf-3 binds ferritin light chain and induces ferritinophagy, resulting in significant increase in the cellular labile iron pool, thereby benefiting iron acquisition by *Ehrlichia*. In this study, we developed Etf-3-specific nanobodies (Nbs) by immunizing a llama with recombinant N-terminal half of Etf-3 (Etf-3N) and C-terminal half of Etf-3 (Etf-3C) and constructing llama Nb cDNA library in pMECS phagemid vector. Nbs were expressed on the surface of VCSM13 phage, and phages that bind Etf-3N and Etf-3C were enriched by panning. After ELISA screening 192 individual anti-Etf-3N Nbs and 384 anti-Etf-3C Nbs expressed in *Escherichia coli* TG1, a total of 124 Etf-3N Nbs with 20 distinct complementarity-determining region 3 (CDR3) sequences, and 42 anti-Etf-3C Nbs with 17 distinct CDR3 sequences were obtained. Subsequently, total 17 Etf-3N and 16 Etf-3C Nbs with distinct CDR3 sequences were cloned into mammalian expression vector. Upon analysis of Nb expression levels in HEK293 cells, 8 each of Etf-3N and Etf-3C were selected for inhibition of *E. chaffeensis* infection by western blot analysis. Based on this analysis, 8 Etf-3N Nbs and 3 Etf-3C Nbs were selected for further analysis of inhibition of *E. chaffeensis* infection by RT-qPCR. The result showed Etf-3N Nbs N48 and N51 significantly inhibit *E. chaffeensis* infection. Co-transfection of RF/6A cells with each of these Nbs with Etf-3-GFP showed their colocalization. Etf-3-GFP transfection induces endogenous ferritin degradation in HEK293 cells, and Nbs N48 and N51 inhibited this process, whereas N58 that did not inhibit *E. chaffeensis* infection, did not inhibit ferritin degradation by Etf-3. Our results demonstrate the development of novel Nbs that inhibit *E. chaffeensis* infection and Etf-3-induced ferritinophagy.

P09

Comparative Genome Sequence Analysis of *Ehrlichia muris* subsp. *eauclairensis* Wisconsin, an Isolate from Human Ehrlichiosis Patients

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In vivo pathogenesis and tick transmission mechanisms of tick-borne zoonoses, human ehrlichioses have been poorly understood, in part due to the lack of defined small animal disease and tick transmission models. *Ehrlichia muris* subsp. *eauclairensis* is the newest member of *Ehrlichia* spp. that causes human ehrlichiosis. Human ehrlichiosis agents also include *E. chaffeensis*, *E. ewingii*, and *E. canis*, but they do not cause progressive infection and overt disease in immunocompetent mice. In contrast, *E. muris* subsp. *eauclairensis* Wisconsin (EmuWisc) causes progressive infection and overt disease in immunocompetent mice. In this study, the complete genome sequence of EmuWisc was determined, and compared with those of related Ehrlichia species causing human and mouse infections, including *E. muris* subsp. *muris* AS145, *E. chaffeensis* Arkansas, and *E. japonica* HF. EmuWisc has a single double-stranded circular chromosome of 1,140,711 bp, which encodes 867 proteins with a similar metabolic potential to other *Ehrlichia* species. EmuWisc encodes homologs of all virulence factors identified in *E. chaffeensis*, including 20 paralogs of P28/OMP-1 family outer membrane proteins, two-component regulatory systems, EtpE invasin, and type I and IV secretion system apparatus and their effector proteins such as *Ehrlichia*-translocated factors and tandem-repeat proteins. Comparative genome sequence analysis revealed conserved and variable features in these virulence factors among *Ehrlichia* species and subspecies. In addition, EmuWisc encodes several species and subspecies-specific hypothetical proteins. The complete genome sequence of EmuWisc will facilitate understanding this under-studied human pathogen and pathogenesis of human ehrlichiosis using the mouse model.

P10

Ehrlichia chaffeensis TRP120-mediated ubiquitination and degradation of the COP9 signalosome

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Ehrlichia chaffeensis modulates numerous host cell processes, including cell signaling, transcription, posttranslational modifications and to promote infection of the mononuclear phagocyte. Modulation of these host cell processes is directed by *E. chaffeensis* effectors, including TRP120. We previously reported that TRP120 moonlights as a HECT E3 Ub ligase that ubiquitinates host cell transcription and fate regulators (PCGF5, FBW7 and ENO-1) which enhances infection and also revealed that *E.chaffeensis* activates the Hedgehog pathway to inhibit apoptosis. In this study, we examined relationship between TRP120 and COP9 signalosome subunit 5(CSN5) that function as deneddylation to inhibit CUL RING E3 Ligases (CRLs) activity. siRNA knockdown of COP9 signalosome coincided with increased *E. chaffeensis* infection and CSN5 degradation was observed during infection. A direct role of TRP120 Ub ligase activity in CSN5 degradation was demonstrated using immunofluorescence microscopy and coimmunoprecipitation and ubiquitination of CSN5 by TRP120 was detected in vivo and in vitro. This investigation further expands the repertoire of TRP120 substrates and extends the potential role of TRP120 Ub ligase in infection by manipulating posttranslational pathway components of the host cell to promote infection.

P11

The structure of the OmpA/Pal protein of *Anaplasma phagocytophilum*

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Peptidoglycan associated lipoprotein (Pal) and Outer Membrane Protein A (OmpA), interact with the outer membrane and peptidoglycan in Gram-negative bacteria, conferring structural integrity to the bacterial cell and functioning in cell division. In addition to these housekeeping functions, some OmpA and Pal proteins have moonlighting roles as virulence factors, facilitating infection and host-pathogen interactions in a range of bacteria. The OmpA-like protein of Anaplasma phagocytophilum (OmpAAp), a tick-borne pathogen that infects a wide range of hosts, seems to function primarily as a virulence factor, since this bacterium lacks a peptidoglycan cell wall. Since OmpAAp binds to host cell surface glycoproteins to facilitate infection, we sought to structurally characterise OmpAAp to understand how it is adapted for host-pathogen interactions. The crystal structure of OmpAAp was solved to a resolution of 1.9 Å and revealed that this protein is similar to Pal but has amino acid insertions that confer flexibility. This insertion is also found in the OmpA-like proteins of other pathogens related to A. phagocytophilum, including genera that synthesize peptidoglycan. OmpAAp also has conserved amino acids for peptidoglycan binding by other Pal/OmpA proteins, suggesting that it retains this ability despite the absence of peptidoglycan. Structural predictions and comparisons with the solved structure of Escherichia coli Pal in complex with TolB, with which it works in concert with during cell division, indicate that OmpAAp could potentially bind to the TolB of A. phagocytophilum but that this binding might be influenced by the flexibility of OmpAAp. Whether the flexibility of OmpAAp is related to its role as a virulence factor and/or is a product of the loss of peptidoglycan in this species are unknowns. Although a co-crystal structure of OmpAAp bound to host sugars could not be achieved, these structures provide a basis for understanding how Anaplasma adhesins and invasins function cooperatively to recognise host cells.

P12

A novel targeted mutagenesis method for generating antisense RNA to knockdown Ehrlichia chaffeensis p28-Outer membrane protein 19 (p28-Omp 19) expression

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Tick-borne diseases resulting from Anaplasma and Ehrlichia species continue to emerge as a major health concern for people, companion, and agricultural animals. Despite some progress, the lack of fully established genetic manipulation methods continues to hinder research in advancing basic and applied research goals. We recently reported the development of targeted mutagenesis methods aiding the disruption of genes in Ehrlichia chaffeensis. Similarly, we reported a targeted gene deletion mutation in Anaplasma marginale. These methods have been helping in multiple ways, including investigating virulence determinants and vaccine development. In the current study, we describe a novel targeted mutagenesis method for creating an antisense mutation in E. chaffeensis to knockdown expression from a highly immunogenic outer membrane protein gene; p28-Omp 19 (ECH_1143), as several attempts to disrupt this gene failed to yield successful mutants (probably because the protein is essential for the bacterial growth). We hypothesized that such genes may be better investigated if a mutagenesis method is developed to reduce the gene expression instead of disrupting the gene function. We, therefore, designed an innovative strategy in creating a mutation to express a short antisense RNA segment complementary to the p28-Omp 19 coding mRNA. We have successfully generated an antisense mutation; the mutation was clonally purified and confirmed the purity by molecular methods. The mutation caused reduction of the bacterial numbers as well as in reducing the mRNA levels relative to 16S RNA, which translated into significant drop in the protein expression. The decline in the p28-Omp 19 in the mutant was compensated with enhanced transcription from its two paralogs: p28-Omp 14 (ECH_1136) and p28-Omp 20 (ECH_1144). The data demonstrate that targeted mutagenesis methods are broadly applicable, including in studies to investigate essential genes of E. chaffeensis.

P13

Characterization of global response regulators of *Ehrlichia chaffeensis*, CtrA and Tr1, is facilitated by targeted mutagenesis to generate C-terminal hemagglutinin tags.

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Ehrlichia chaffeensis is a tick-transmitted rickettsial causing infections in people and various vertebrates. It has a biphasic developmental cycle in vertebrate monocytes/macrophages and tick cells. It is unclear how *Ehrlichia* species regulate gene expression supporting biphasic development and adaptation to dual hosts. *E. chaffeensis* has a reduced genome with only a few genes coding for DNA binding proteins (DBPs). CtrA is a known master regulator in bacterial cell cycle regulation. Although CtrA is shown to regulate stress response and Tr1 contributes to the differential expression of p28 OMPs, the functioning landscape of two regulators remains elusive. We created hemagglutinin (HA) gene sequence tag-inserted mutations to generate C-terminal fusion proteins of CtrA and Tr1 (CtrA-HA and Tr1-HA) by performing mutagenesis experiments as per our recently defined protocols; the presence of targeted mutations and fusion protein expression were verified. We then used the mutant bacteria to perform chromatin immunoprecipitation using an anti-HA antibody followed by ultra-high-throughput DNA sequencing (ChIP-Seq). CtrA and Tr1 bound to 337 and 448 *E. chaffeensis* genomic locations, respectively. CtrA and Tr1 binding sites were located primarily in intergenic sequences (80%). About 50% overlap in the DNA binding domains was observed between the CtrA and Tr1 (182 binding peaks overlapped), suggesting that the two DBPs probably cooperatively regulate gene expression. We validated the ChIP-Seq data by performing gel mobility shift experiments using 19 and 20 DNA fragments randomly selected from peak sequences identified from the ChIP-Seq data for CtrA and Tr1, respectively. This study suggests that CtrA and Tr1 impact several biological processes of *E. chaffeensis*, including protein secretion and transport, lipoprotein metabolism, the expression of several outer membrane proteins and DNA replication.

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P14

Unfolding vector immunity and advancing research tools for *Ixodes scapularis*

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Ticks are responsible for 91% of vector-borne disease cases reported to the CDC in 2019. The North American deer tick, *Ixodes scapularis* is a significant transmitter of various human pathogens, including Lyme disease and human granulocytic anaplasmosis. We have limited knowledge about what factors influence vector competency, which is defined as the ability of an arthropod to harbor and transmit pathogens. Arthropod immunity impacts vector competence, but the immune system in non-insect arthropods is poorly understood. Because innate immune processes have been linked with cellular stress responses, we investigated the relationship between the unfolded protein response (UPR) and innate immunity in ticks. The UPR is a highly conserved mechanism across species and is regulated by three transmembrane receptors, with IRE1α being the most conserved. We found that key UPR genes, IRE1α and TRAF2, are upregulated in infected ticks and demonstrated the activation and interaction of the two proteins. *In vitro* and *in vivo* findings show both IRE1α and TRAF2 limit bacterial colonization of *A. phagocytophilum* and *B. burgdorferi*. This linkage leads to the induction of the Immune Deficiency (IMD) pathway NF-κB-like factor, Relish. The molecular players linking IRE1α-TRAF2 signaling with the IMD pathway are unknown. To address this question, we adapted a proximity-based protein labeling assay for use in tick cells that fuses an engineered soybean ascorbic peroxidase (APEX2) to *Ixodes* TRAF2. By identifying TRAF2 interactions we will uncover the mechanistic linkage between the UPR and the IMD pathway thereby providing novel insights into vector competency.

P15

The blood microbiome of patients with acute febrile illness in the Mnisi community, South Africa, reveals the presence of a novel member of the family *Rickettsiaceae*

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Febrile illness is a major cause of morbidity and mortality in resource-poor countries and a common reason for people to seek medical care in Africa. In sub-Saharan Africa, vector-borne pathogens such as Rift Valley fever virus, *Borrelia* spirochetes (tick-borne relapsing fever), and *Rickettsia africae* (African tick bite fever) are increasingly implicated in acute febrile illness (AFI) in rural, pastoral, and mixed farming communities; such AFI cases are often clinically misdiagnosed as malaria. There are few reports on the epidemiology of non-malarial AFI aetiologies in Africa; the true burden and causes of such illnesses is unknown. This study was aimed at identifying potential aetiological agents of AFI in patients presenting at the Hluvukani Clinic, the main clinic serving the Mnisi community located at a human-wildlife-livestock interface in Bushbuckridge, Mpumalanga Province, South Africa. Samples were collected from 196 patients with non-malarial AFI and 26 apparently healthy individuals. DNA was extracted and the bacterial microbiome was examined by amplifying the 16S rRNA gene (V1-V9) using universal 16S rRNA barcoded primers. 16S amplicons were sequenced using circular consensus sequencing on a PacBio sequencing platform. Preliminary results revealed a novel 16S rRNA gene sequence belonging to the family *Rickettsiaceae* in 13 AFI patient samples. This sequence shares 89.4% 16S rRNA sequence identity with *Rickettsia rickettsia*, 87.6% with *Occidentia massiliensis* and 88.1% with *Orientia tsutsugamushi*. Phylogenetic analysis confirmed the phylogenetic position of the sequence in the family *Rickettsiaceae* and suggests that it represents a new genus ancestral to *Rickettsia*, *Orientia* and *Occidentia*. This study highlights a potential expansion of the family *Rickettsiaceae* and the need for more research to thoroughly define the novel organism and its importance as a disease agent.

P16

An anti-vector vaccine targeting rickettsial pathogen transmission from ticks

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In the United States, human anaplasmosis caused by *Anaplasma phagocytophilum*, is one of the most common arthropod-borne diseases. In humans, *A. phagocytophilum* persists within neutrophils primarily by combating oxygen-dependent killing mechanisms. While much is known about the interactions of *A. phagocytophilum* with mammalian cells, very little is known about how *A. phagocytophilum* survives within the medically important arthropod tick vector, Ixodes scapularis. Previous studies from my laboratory showed that *A. phagocytophilum* activates *I. scapularis* organic anion transporting polypeptide 4056, *isoatp4056*, and kynurenine aminotransferase (KAT), an enzyme that catalyzes the formation of Xanthurenic acid (XA) in the tryptophan metabolism pathways for its colonization in ticks. Using RNAi, we reported an important role for *isoatp4056* during *A. phagocytophilum* survival and transmission from ticks to the murine host. We noted that *A. phagocytophilum* induces XA levels in ticks and tick cells and inhibits reactive oxygen species (ROS) and cell death via p38 MAPK facilitating bacterial and tick survival. Recently, we identified that tick microRNA 133 (*miR133*) targets *isoapto4056* mRNA. *Anaplamsa phagocytophilum* downregulates *miR133* to upregulate *isoatp4056* expression during transmission from ticks to the vertebrate host. Microinjection of *miR133* into ticks impaired *A. phagocytophilum* transmission from infected ticks. The current study provides evidence that targeting IsOATP4056 with an anti-vector vaccine affects the transmission of *A. phagocytophilum* from ticks to the vertebrate host. Passive immunization, tick-mediated transmission, and in vitro studies performed with mice and with tick cells showed that EL-6 antibody not only impairs *A. phagocytophilum* transmission from ticks to the murine host but also aids in the reduction in the bacterial loads within engorged ticks and in tick cells by activation of arthropod Toll pathway. Collectively, these results provide evidence for the development of an anti-tick vaccine to target the transmission of *A. phagocytophilum* and perhaps other rickettsial pathogens of medical importance.

P17

Oral delivery of dsRNA for RNAi via capillary feeding and artificial membrane feeding

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RNAi assays are an important tool for tick and tickborne pathogen research. The current gold standard for RNA delivery in RNAi studies is micro-injection with oral delivery of RNA being a relatively new and novel way to deliver RNA constructs into ticks. However, micro-injection is highly labor and skill intensive which limits sample sizes in tick studies. This study expands on current oral delivery methods of capillary feeding and investigates the potential of membrane feeding as an alternative oral delivery method. We examined how different concentrations of dsRNA affected gene knockdown in *Ixodes scapularis* fed via established capillary feeding techniques to build an understanding for the dose response of dsRNA RNAi assays. Using this dose response data, a minimum concentration of dsRNA to achieve sufficient gene knockdown was identified. Using this minimum concentration amount, dsRNA will be mixed into blood and delivered to feeding ticks via artificial membrane feeding. Gene knockdown of these membrane fed treated ticks will be evaluated against controls and capillary fed treated ticks. Concurrent delivery of dsRNA and feeding of ticks will help simplify RNAi experiments by allowing feeding and RNA delivery in one experimental step and facilitate experiments with tick species and life stages that require large hosts unavailable in many labs.

P18

PERK-mediated antioxidant response is key for pathogen persistence in ticks

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Ticks are prolific spreaders of pathogens that plague human and animal health. A crucial phase in the tick-borne pathogen lifecycle is the time spent colonizing and persisting within the arthropod between bloodmeals. While many factors impact the way transmissible pathogens interface with the arthropod, a key force shaping this interaction is tick immunity. Recently, the unfolded protein response (UPR) has been linked to arthropod immunity. The IRE1α branch of the UPR pairs with TRAF2 to activate the IMD pathway and potentiate reactive oxygen species (ROS). This signaling network functionally restricts Anaplasma phagocytophilum (granulocytic anaplasmosis) and Borrelia burgdorferi (Lyme disease) within the tick. As vector immunity continues to be explored, a fundamental question has emerged: how are tick-borne pathogens persisting in the arthropod despite immunological pressure? We found that B. burgdorferi and A. phagocytophilum also trigger the UPR receptor PERK. Persistent infection activates the central regulatory molecule, eIF2α, in Ixodes scapularis ticks through PERK, which induces the antioxidant response regulator, Nrf2. Disabling the PERK-eIF2α pathway in tick cells caused sustained ROS and reactive nitrogen species (RNS) production and enhanced microbial killing. Knocking down the PERK-eIF2α pathway in vivo significantly inhibited A. phagocytophilum and B. burgdorferi tick colonization and reduced the ability of the microbes to persist through the molt. Overall, we have uncovered a mechanism at the vector-pathogen interface that promotes persistence of transmissible microbes despite active immune assaults.

P19

Detection of *Candidatus* Rickettsia andeanae in South Carolina

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Globally, ticks are significant vectors of bacteria, viruses, and parasites which may cause disease and death in animals and humans. In the United States of America, there are 29 recognized spotted-fever group *Rickettsia* (SFGR) transmitted by *Dermacentor*, *Rhipicephalus*, *Haemaphysalis*, and *Amblyomma* ticks. Both *Amblyomma maculatum* Koch and *Dermacentor variabilis* Say are three-host tick vectors of pathogenic *Rickettsia* spp. with recent geographic range expansion, making these two tick species of public health concern. South Carolina is particularly susceptible to shifts in the spatial distribution of ticks because of climatic changes and steady population increases which have led to rapid development. *Candidatus* Rickettsia andeanae is a spotted fever group Rickettsia of undetermined pathogenic potential not previously reported in South Carolina. From 2020-21, the Laboratory for Vector-Borne and Zoonotic Diseases received host-attached ticks from 14 animal shelters representing all four public health regions of the state. A total of 792 tick specimens from humane animal shelters were tested for SFGR pathogens, and sequencing revealed an overall Ca. *R. andeanae* prevalence of 0.90%. Species-specific prevalence was 3.46% (*A. maculatum*) and 3.45% (*D. variabilis*). Except for a single tick, for both years across all sites, ticks positive for Ca. *R. andeanae* were collected off host animals in August and September. Whether this novel *Rickettsia* spp.is an insight into existing microecology or an emerging pathogen of interest, it is nevertheless useful in understanding the not fully elucidated dynamics of vector symbiosis and tick-borne disease transmission.

P20

Secretion and biological roles of a hemolysin, tlyC2, in rickettsial intracellular lifecycle

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Spotted fever group (SFG) Rickettsia species display obligate intracellular lifecycle and cause tick-borne rickettsiosis worldwide. Over the past two decades, the reported cases of tick-borne rickettsiosis have been rising, partly due to the environmental changes that support the expansion and invasion of ticks into new geographical areas. Recent advances in developing genetic tools for Rickettsia provide unique opportunities to identify molecular effectors and characterize their biological roles in host-pathogen-vector interactions. As an obligate intracellular pathogen, Rickettsia has evolved to reside within the cytosolic compartment of vascular endothelial cells and spread to neighboring cells without eliciting host immune responses during the early phase of infection. Previous work suggests that typhus group Rickettsia, but not SFG Rickettsia, synthesizes a hemolysin that allows the microorganisms to escape vacuolar structures. However, our bioinformatic analysis identified three conserved hemolysins in both SFG and TG Rickettsia, implicating that these gene products may have essential functions in the rickettsial intracellular lifecycle. Thus, we hypothesize that SFG Rickettsia synthesizes hemolysins and regulates the secretion of hemolysins during the intracellular lifecycle. Using a kkaebi transposon mutant library, we demonstrate that TlyC2 is synthesized and deposited on the outer membrane of Rickettsia and potentially transferred to the host cell membrane during the invasion of host cells and escape to neighboring cells. In addition, we report that TlyC2 is essential for hemolytic activities in a manner dependent on host species, temperature, and pH. Our studies support the model where Rickettsia synthesizes conserved hemolysins to perturb host cell membrane structures during the initial invasion, evasion from autophagic responses, and spread to neighboring cells. Additional studies are warranted to gain further insights into the biological roles of rickettsial hemolysins in pathogenesis and transmission.

P21

Contributions of the Ixodes scapularis endosymbiont Rickettsia buchneri to infection and immunity of its tick host

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Ixodes scapularis is the major vector of tick-borne disease in North America, transmitting seven known human pathogens. However, despite its capability as a vector and its geographical overlap with other ticks that host pathogenic spotted fever group rickettsiae (SFGR), *I. scapularis* transmits no disease-causing *Rickettsia*, and instead is widely infected by the endosymbiont *Rickettsia buchneri*. Presence of this symbiont has been proposed as the primary reason that *I. scapularis* is rarely infected by nor transmits pathogenic members of the SFGR. The *R. buchneri* genome encodes putative antibiotic synthesis operons and a toxin/antidote module, which may contribute to *R. buchneri*'s role in preventing rickettsial superinfection of its tick host. However, beyond bioinformatic analyses, no investigation of the antibiotic activity of the products of these genes has been attempted. We previously showed, using tick cell culture, that presence of *R. buchneri* prevented infection with the human pathogen *R. parkeri*, and reduced infection by *Anaplasma phagocytophilum* and *R. monacensis*. Beyond the potential effect of the encoded genes for antibacterial antagonism, we also hypothesize that the endosymbiont plays a role in modulating the immune response of its tick host to reduce infection by tick-borne pathogens. Both these mechanisms driven by *R. buchneri* may play important roles in determining the tick's vector competence for human-pathogenic bacteria and shaping tick borne disease epidemiology.

Using qRT-PCR, the expression of *R. buchneri*'s putative antibacterial antagonism genes was analyzed in tick cells challenged with different pathogens, and in various tick life stages. The expression of genes from multiple immune pathways of the tick host were also examined in the presence and absence of the symbiont. These results will clarify to what extent *R. buchneri*'s antibacterial machinery is employed during tick infection, and whether the symbiont is involved in shaping *I. scapularis* immunity.

P22

Cell polarity of *Rickettsia parkeri*

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The morphology and subcellular organization of bacterial cells are central to their ability to replicate and to sense and interact with their environments. Most of our knowledge of bacterial cell biology comes from studies in free-living models. Notably, the cell biology of obligate intracellular bacteria, including members of the Rickettsiales, is relatively poorly understood. To bridge this gap, we have developed imaging-based approaches to understand morphogenesis and cellular organization of *Rickettsia parkeri*, an obligate intracellular, tick-borne human pathogen in the Spotted Fever Group of the *Rickettsia* genus. Specifically, we developed approaches to measure morphology, growth kinetics, and protein localizations of live *R. parkeri* in infected host cells. We are now applying these methods to investigate poorly understood aspects of *R. parkeri* cell biology. This includes cell polarity, or the ability to target molecules or structures to one or both ends of the cell. Polarity is leveraged by diverse bacteria to promote cell fate asymmetry, adhesion, motility, environmental interactions, and other processes. Despite a lack of obvious morphological polarity, *R. parkeri* and other Rickettsiales encode homologs of the cytoplasmic polar organizing protein PopZ. In other bacteria, PopZ forms a phase-separated polar hub that recruits numerous client proteins and directs diverse polarized processes. We found that a fluorescent fusion to PopZ localizes to one or both poles in *R. parkeri*, reflecting apparent cell cycle regulation of its localization and function. Moreover, we found that disrupting cell polarity through loss of rod shape eliminated formation of a polarized actin comet tail required for actin-based motility of *R. parkeri*. We are currently pursuing the hypothesis that PopZ mediates polarization of processes central to *R. parkeri* replication and interactions with the host during infection. We expect our findings to unveil novel mechanisms required for rickettsial replication that may be targeted to control bacterial growth.

P23

Calcium signaling in spotted fever group rickettsiae induced microvascular endothelial cell barrier permeability

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Spotted fever group rickettsiae (SFGR) are obligate intracellular bacteria that parasitize cellular ATP while causing severe disease dominated by increased vascular permeability (VP), the major pathophysiological effect of SFG rickettsioses. In physiologic and pathophysiologic conditions, microvascular endothelial cell (MEC) VP is linked to increased intracellular calcium concentrations $[Ca^{2+}]_i$ ordinarily regulated by ATP-dependent calcium channel pumps. We used *Rickettsia parkeri* (RP)-infected human brain MECs (hBMECs) treated with L-, N-, and T-type voltage-gated calcium channel (VGCC) blocker benidipine and found a benidipine dose-dependent delay in RP-induced MEC barrier permeability. We hypothesize that SFGR infection modulates specific calcium transport systems leading to barrier dysfunction before direct rickettsial MEC damage. To confirm which calcium channels are expressed in hBMECs, RNA-seq demonstrated expression of alpha pore-forming subunit transcripts for L-($Ca_v1.3$) and T-($Ca_v3.1$) type VGCCs, targets of benidipine. RP-infection decreased hBMEC ATP levels ($p<0.001$) in bioluminescence assays associated with decreased hBMEC barrier permeability detected by electric cell-substrate impedance sensing. Using Ca^{2+}-sensitive dye Fluo-4-AM, $[Ca^{2+}]_i$ increased in RP-infected hBMECs ($p=0.001$), and this correlated with decreased hBMEC barrier permeability. Notably, benidipine abrogated ATP decline ($p=0.001$) and RP-induced hBMEC $[Ca^{2+}]_i$ increases ($p<0.01$) corresponding to delayed RP-induced hBMEC barrier dysfunction. Benidipine delayed RP cell-to-cell spread and growth in a dose-dependent manner, findings linked to inhibition of rickettsial actin-tail formation with benidipine. SFGR-induced MEC permeability involves reduced cellular ATP accessible to calcium pumps/channels, consequently increasing $[Ca^{2+}]_i$ and hBMEC barrier dysfunction that can be abrogated by blocking calcium flux at benidipine-sensitive VGCCs. How this impacts rickettsial actin-tail formation and thus cell-to-cell spread is not known but suggests a rickettsia-specific channelopathy for VP pathogenesis. Whether this results from simple ATP depletion and VGCC dysfunction or a specific targeting of VGCCs by rickettsiae needs to be investigated.

P24

Phenology, host associations, and prevalence of *Rickettsia parkeri* in *Amblyomma maculatum* group ticks collected in southern Arizona.

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Rickettsia parkeri is a spotted fever group *Rickettsia* transmitted by ticks in the Americas. Clinical cases have recently been identified in Arizona and have been associated with *Amblyomma maculatum* group ticks present in, or proximal to semi-arid mountainous regions in the southwestern USA and Mexico. Prior to 2022, only a few published records reported collection of immature stages, including a single larva, all removed from birds. At present, the ecology of this tick is not well understood in this region.

Beginning in August 2022, monthly small mammal trapping sessions were conducted in Cochise County, AZ at locations where questing adult *A. maculatum* s.l. had previously been collected. Ear tissue, blood, and ectoparasites were obtained from anesthetized hosts. Passerine birds were mist-netted at the same sites during winter and spring months and checked for ectoparasites. Ear tissue, blood and ticks were screened with a pan-*Rickettsia* PCR assay and positives were subjected to further species-specific assays. A subset of replete larvae and nymphs were allowed to molt for clear morphologic identification. Immature stages of *A. maculatum* s.l. were collected from rodents and birds in each month sampled, and both *R. parkeri* and *R. andeanae* DNA were detected in rodent tissue. We report a preliminary account of the phenology, host association and presence of *Rickettsia* spp. in *A. maculatum* s.l. in a region where *R. parkeri* rickettsiosis is an emerging disease.

P25

Ticks and tick-borne pathogens in green zones of a university campus in Georgia, USA

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Tick encounters with people are typically associated with open forest environments and grassy outdoor spaces; however, questing ticks can also be found in green spaces in urban areas. The purpose of this study was to perform surveillance of ticks and tick-borne pathogens (TBP) in green and forested areas along walkways of a university campus.

Ticks were collected by flag-dragging seventeen sites weekly between June 2022 and April 2023. Each tick was identified to species, life stage, and sex using standard keys. Ticks were surface disinfected, their DNA was extracted and tested by SYBR-Green PCR for spotted fever group rickettsiae (SFGR) and *Anaplasmataceae* sp. followed by typing using restriction fragment length polymorphism analysis (RFLP) and amplification of genomic genus-specific sites with variable numbers of tandem repeats (VNTR).

447 ticks were collected from 12 of 17 (70.6%) sites surveyed: 135 adults and nymphs of *Amblyomma americanum* (30.3%) and 41 adult *Ixodes scapularis* (9.2%). All larvae were *Amblyomma* species. *A. americanum* was collected during warm weather months, June through October and again starting March ($r=0.56$, $p<0.01$) whereas *I. scapularis* was found in November through February. SFGR DNA was detected in 41.6% (n=135) of *A. americanum*, 11.8% (n=17) of *I. scapularis*, and 43.2% of 35 *Amblyomma* larva pools (5 to 8 larvae per pool) tested. Only DNA of *Rickettsia amblyommatis* was identified by RFLP in all positive *Amblyomma* ticks. Several genotypes of *R. amblyommatis* were identified based on pMCE-2 VNTR typing. Five *Anaplasmataceae* positive *A. americanum* contained *Ehrlichia chaffeensis* as determined by amplification of the VLPT fragment. *I. scapularis* were positive for *R. buchneri.*

Further testing is needed to characterize the genetic diversity of other *Anaplasmataceae* detected. These findings clearly demonstrate that there is a potential risk of tick-borne infection for individuals traversing university properties.

P26

Comparative analysis of Rickettsia actin-based motility mechanisms

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After invading host cells and gaining access to the cytoplasm, many *Rickettsia* species undergo actin-based motility through the activity of two bacterial proteins, RickA and Sca2. For the spotted fever group species *R. parkeri*, actin-based motility occurs in temporally distinct phases, with RickA driving motility <1 h post infection and Sca2 driving motility >8 h post infection. The ancestral and non-pathogenic species *R. bellii* expresses orthologs of RickA and Sca2, but how each contributes to the mechanism and timing of motility remains unclear. We examined the timing of *R. bellii* actin-based motility in infected human epithelial and endothelial cell lines. In contrast with *R. parkeri*, *R. bellii* only initiated motility >4 h post infection. Sca2, but not RickA, consistently localized to the pole of *R. bellii* with actin tails, suggesting that Sca2 is the primary motility effector. Moreover, *R. bellii* exhibited distinctive actin tail morphologies as assessed by fluorescence microscopy. In epithelial cells, *R. bellii* actin tails were thinner and curvier than *R. parkeri* tails. In endothelial cells, *R. bellii* actin tails were shorter and more splayed than for *R. parkeri*. To further compare the ultrastructure of *Rickettsia* actin tails, we have initiated cryo-electron tomography studies. In initial observations of *R. parkeri* actin tails, individual actin filaments were resolved as they emanate from the bacterial pole, allowing for a high-resolution reconstruction of the actin filament network. In parallel, biochemical characterization of purified proteins revealed *R. bellii* RickA activated the host Arp2/3 complex. The molecular mechanism of *R. bellii* Sca2 is under investigation. These data suggest that RickA and Sca2 orthologs from diverse *Rickettsia* species polymerize actin and drive movement, yet species-specific differences result in variations in the timing and mechanisms of actin-based motility. Further analyses will shed light on the evolution of motility mechanisms and their role in pathogenicity.

P27

The Role of Endosymbiotic Rickettsia spp. in Spotted Fever Group Infections

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Spotted Fever Group (SFG) Rickettsia are obligate intracellular bacterium typically spread though the bite of a tick. The virulence of these bacteria ranges from mild illness with little pathology to severe infections with high mortality rates. Coinfections with Rickettsia was historically considered unlikely, as one species would interfere and block another, commonly referred to as priority effect. Recent reports, however, have observed wild caught ticks being infected with multiple species of Rickettsia with varying virulence; this leaves the role of priority effect in question. To address this, the long-term goal of this study is to observe changes in pathogenesis during coinfections between Rickettsia of varying virulence. For this study, R. bellii was selected as our low virulence species since it has been found within ticks along with other Rickettsia spp. In this work, an increase in cytotoxic activity was observed in Vero cells when infected with R. bellii in a dose and time dependent manner. The next step in this study is to determine how R. rickettsii impacts growth of R. bellii in Vero cells as well as tick and mammalian models. With the spread of their vectors into the new areas, which subsequently bring these pathogens with them, understanding how these coinfections may facilitate or antagonize pathogenesis is important for public and veterinary health.

P28

Dermacentor similis is a competent reservoir of *Rickettsia rickettsii* under laboratory conditions

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Recently, the discontinuous western United States populations of *Dermacentor variabilis*, a vector of the medically important pathogens *Rickettsia rickettsii* and *Francisella tularensis*, were redescribed as a new species (*Dermacentor similis*) based on morphological and molecular characteristics. While the differentiation helps clarify the taxonomy of *Dermacentor* spp. ticks, it leads to new questions surrounding the vector competence and public health import of the newly described species. The majority of vector competency work involving *Rickettsia rickettsii* and other tick-borne pathogens in *Dermacentor variabilis* has been performed using eastern populations, which is considered *D. variabilis* sensu stricto. Here, we assess the ability of a population of *D. similis* from California to acquire and maintain *R. rickettsii* through the molt after exposure to guinea pigs (*Cavia porcellus*) that had been needle-inoculated with *R. rickettsii*. Two strains of the pathogen (Di6 and Sheila Smith) were inoculated and assessed separately. Subsequently, we compared the ability of infected females to successfully transovarially transmit *R. rickettsii* to their larval progeny via detection of pathogen-specific DNA in larval clutches. Our findings show that *D. similis* is capable of nymphal to adult stage transstadial transmission of *R. rickettsii*; successful transstadial transmission ranged from 0-100% (mean 65.9%). *Dermacentor similis* females were also able to successfully transovarially transmit *R. rickettsii* (mean 6.4%, range: 0-10%). Notably, the strain of *R. rickettsii* appeared to affect the success of transmission, with the Di6 strain having lower transstadial (mean 42.3% versus 89.4%) and transovarial transmission (mean 0% versus 9.6%) than Sheila Smith.

P29

Development of an intradermal model of infection in guinea pigs to investigate the pathogenic potential of *Rickettsia* species

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Worldwide, nearly 20 species of *Rickettsia* are recognized as human pathogens. While some other members of this genus are considered nonpathogenic endosymbionts, the pathogenic potential of many species remains uncharacterized. Various methods have been employed to investigate the pathogenicity of rickettsiae; the most common uses an animal model of infection to recapitulate rickettsiosis. Guinea pigs are often used for these studies due to their physiological and immunological similarity to humans. Inoculation of rickettsiae into guinea pigs using an arthropod vector is the ideal method for animal studies, though needle inoculation is often used, primarily through an intraperitoneal route. Intraperitoneal inoculation has been successfully used in studies investigating the pathogenic potential of rickettsiae, but this method does not approximate natural transmission of *Rickettsia* into the skin through the bite of an arthropod vector. This study was initiated to develop a reliable intradermal model of infection in guinea pigs that more closely resembles the natural route of rickettsial infection. Using *R. parkeri* Black Gap as a control and *R. tillamookensis* as the experimental agent, we compared infection of guinea pigs using both intraperitoneal and intradermal inoculation of *Rickettsia*. Results of the study revealed no significant differences in outcomes between the two inoculation methods; both groups exhibited orchitis and rickettsial dissemination to the ear, though only the intradermally inoculated group displayed low-grade fever. Histopathology of intradermally inoculated animals revealed lymphohistioctyic inflammatory cell infiltrates in the deep dermis at inoculation sites. Surprisingly, no rickettsiae were identified by immunohistochemical staining at these sites five days post-inoculation, and no intradermally inoculated animals developed an eschar. This intradermal inoculation model shows promise for use in analyzing pathogenic potential for uncharacterized *Rickettsia* species; however, additional studies are needed to refine the model, including validation using a *Rickettsia* species with more thoroughly characterized clinical outcomes in guinea pigs.

P30

Specific Binding of anti-Rickettsia Immune IgM to the β-peptide of rOmpB

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While studying our printed poster before the previous meeting in Greenville I noticed an oddity in the ELISA IgM results. For each of 18 different SFG species the ELISA antigen was a heteroduplex eluted from the s-layer, namely a twisted combination of rOmpA and rOmpB. With around half of the results producing the same positive value and the remainder being totally negative, this might just be rOmpA blocking reactive sites. After talking with Greg Dasch we trypsinized these heteroduplexes and on IgM western blot found a single 25 kDa band (with a lower molecular weight dot) on all SFG and TF (except R. felis). Suspecting this to be the highly conserved β-peptide (32 kDa) we began preparing a recombinant to work further with. While waiting we arranged an in vitro situation using an SFG strain with PMA-treated THP-1 cells (macrophages) and a series of different human sera added. We included IgG positive and negative and IgM positive and negative to these SFG cultures. During the 3 days in culture, the odd ones were the IgM positive wells where the Rickettsia were clustered both free (most) and attached to the macrophages, but the macrophages had many fewer internal forms (phagocytized). We will be collaborating with academic researchers to fit this new property into the pathogenic capabilities of these pathogens.

P31

CRISPR-Cas9 Gene Editing in Tick Cells

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Ixodes scapularis is a medically important chelicerate that transmits bacteria, viruses, and protozoa to humans and other animals. Although I. scapularis is responsible for the majority of vector borne diseases in the United States, there is a lack of genetic tools for molecular research due to the substantially longer life cycle and unique biology of this ectoparasite. Here, we developed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based technologies for the I. scapularis ISE6 cell line. We generated a strategy for CRISPR-Cas9 gene editing by testing different approaches, including plasmid or ribonucleoprotein transfection and non-homologous end joining or homology dependent recombination. We targeted the E3 ubiquitin ligase X-linked inhibitor of apoptosis (xiap) of the immune deficiency (IMD) pathway, which affected colonization of the rickettsial agent Anaplasma phagocytophilum. We also developed a methodology for inducing tick gene expression from endogenous loci using CRISPR activation (CRISPRa). CRISPRa utilizes the catalytically dead Cas9 (dCas9) enzyme conjugated with transcriptional activators and single guide (sg)RNA. Currently, we are improving the CRISPR activation system by testing the VP64-p65-Rta (VPR) and Synergistic Activation Mediator (SAM) methodologies. Finally, we are identifying RNA polymerase II and III promoters for enhancing the expression of Cas9 and sgRNAs in the I. scapularis ISE6 cell line. Collectively, we made an important technological advancement in tick molecular biology, which will aid in understanding I. scapularis-microbe interactions.

P32

Role of $\gamma\delta$ T-cells in *Rickettsia parkeri* infection at skin interface

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Rickettsia parkeri, an emerging bacterial pathogen, is transmitted by *Amblyomma maculatum* via infected tick saliva. During feeding, ticks secrete numerous salivary factors manipulating the host's hemostatic and immune response to promote blood feeding. With immunomodulatory properties, tick saliva inhibits and alters multiple immune cell functions, including neutrophils, macrophages, and dendritic cells, likely facilitating *Rickettsia* transmission. To counteract the tick salivary and bacterial factors, host cutaneous resident immune cells provide initial protection. Skin resident γδ T-cells, a subset of the T-cell population, are crucial for maintaining skin homeostasis and preventing skin infection. However, the role of γδ-T cells in controlling *R. parkeri* infection in vertebrate hosts remains unknown. Utilizing wild-type (WT) and γδ T-cells knockout (TCRδ KO) mice, *R. parkeri*-infected nymphs were fed for 3, 5, and 7 days to assess bacterial transmission. Rickettsial DNA was detected at the tick attachment site over the course of feeding, and the highest bacterial burden was measured 5 days post-infestation in both WT and TCRδ KO skin samples. However, no significant differences were observed in rickettsial load and tick weight between the experimental groups at all time points. Furthermore, to compare immune cells recruited to the bite site, immunostaining, flow cytometry, and single cells RNA sequencing will be employed to characterize the host immune response in the early phase of rickettsial infection when delivered by the natural, tick inoculation.

P33

LncRNA-mediated regulation of endothelial inflammation during rickettsial infection

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Pathogenic Rickettsia species, including R. rickettsii cause severe infections in humans, characterized by endothelial activation and dysfunction, resulting in inflammation, pulmonary/cerebral edema, collectively termed as vasculitis. Long non-coding (lnc) RNAs, longer than 200 nucleotides, transcribed by RNA polymerase II, are functional RNA molecules, involved in the regulation of important cellular functions and intricately linked to the determination of innate and adaptive immune responses. To determine the expression profile of lncRNAs, we infected human dermal microvascular endothelial cells (HMEC-1) with R. rickettsii (strain Sheila Smith) for 3 and 24h. Interestingly, the Nuclear paraspeckle assembly transcript 1 (NEAT1) was significantly upregulated in rickettsiae-infected endothelial cells (EC) as compared to mock- infected cells. Considering the concepts of endothelial heterogeneity, lncRNA expression specific to tissues, and pulmonary and cerebral edema as the prominent pathogenic features of rickettsial infections, we next quantified NEAT1 expression in human pulmonary and cerebral EC. The results demonstrate that NEAT exhibited higher expression in both lung and brain EC during rickettsial infection as compared to mock-infected cells. Toll like receptors (TLRs) are considered as primary sensors of microbial pathogens; therefore, we measured the expression of both TLR2 and TLR4 in EC during rickettsial infection and the results clearly suggest that both receptors are activated at 3h and 24h post-infection. Because enhanced expression of pro-inflammatory cytokines, chemokines, and interferons is an important feature of infected endothelium, we further examined the expression levels of IL-6, IL-8 and IFN-β expression 24h post infection. Our data indicate increased endothelial expression of cytokines during infection, and that Interferon-beta (IFN-β) expression is regulated by NEAT1. Ongoing studies on determining the regulatory potential of NEAT1 in the molecular circuitry underlying regulation of inflammation and host immunity will enhance our knowledge of context-specific physiological roles of NEAT1 in the determination of host responses to pathogenic rickettsiae.

P34

Characterizing the functional antibody response to *Anaplasma marginale*

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Bovine anaplasmosis is caused by *Anaplasma marginale*, a tick-borne intra-erythrocytic bacterium that causes acute hemolytic anemia and a significant reduction in cattle production globally. Currently, no safe and effective vaccines are available to prevent this disease. Infected cattle that survive acute infection mount a strong antibody response and control the pathogen at levels below detection. IgG, and particularly opsonization with IgG2 and macrophage phagocytosis are likely important for immune protection. However, large knowledge gaps remain in our understanding of the immune effectors involved in pathogen control.

IgG has two major domains. The Fab domain binds the antigen and provides specificity to the antibody response. The Fc domain binds Fc receptors (FcRs) present on most immune cells, and although often considered invariant, it has heterogeneity based on the antibody subclass and glycosylation of the CH2 domain of the heavy chain. Together the engagement of different Fc domains with different classes of FcRs results in the triggering of diverse effector responses. Many of these responses, including monocyte and neutrophil phagocytosis, γδ+ T cell activation, complement fixation, and platelet activation, could play a role in anti-*A. marginale* immunity.

To start to identify these effector responses, we have developed Fc-dependent neutrophil and monocyte phagocytosis assays in cattle. We hypothesize that: 1) there is greater Fc-mediated monocytic phagocytosis as compared to neutrophilic phagocytosis in immune sera from cattle at the time of control of *A. marginale*; 2) the magnitude of the Fc-mediated phagocytic responses varies depending on the *A. marginale* antigen.

We will present these results in the context of the development of this platform to help us define immune correlates of protection against bovine anaplasmosis during natural infection and vaccination and the use of these correlates to develop a safe and effective anti-*A. marginale* vaccine.

P35

Benidipine delays disease severity and immunopathogenesis in *Anaplasma phagocytophilum* infection in the AG129 mouse model

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Anaplasma phagocytophilum causes febrile illness often characterized by severe inflammatory complications (sepsis, acute respiratory distress syndrome, hemophagocytic lymphohistiocytosis/macrophage activation) likely associated with microvascular endothelial cell (MEC) barrier compromise. Inflammatory cell activation and MEC barrier permeability are inevitably linked as a result of changes in intracellular calcium concentrations $[Ca^{2+}]_i$. We previously showed in vitro that *A. phagocytophilum*-infected HL-60 cells caused a dose-dependent compromise in MEC barriers as measured by electric cell impedance sensing, and this is abrogated by the calcium channel blocker benidipine. We hypothesize that benidipine reduces vascular permeability and tissue inflammatory cell infiltration in vivo by controlling Ca^{2+} flux in animal models of *A. phagocytophilum*. AG129 mice that lack IFN receptors yet attain significant inflammatory changes and high bacterial loads, were mock-infected or infected IP with 10^6cell-free *A. phagocytophilum* and were simultaneously treated with, 1, 3, or 10mg/kg/d benidipine or vehicle-only. Animals were sacrificed on d7 and d10 post-infection to examine clinical signs, histopathologic changes, dextran extravasation, and bacterial load. No clinical signs or adverse treatment effects were observed. Infected mice receiving 1 or 3mg/kg/d benidipine gained more weight than vehicle-only controls. All infected animals treated with any benidipine had less splenic enlargement. Bacterial loads were lower only on d7, in liver ($p=0.0052$) of infected mice treated with 10mg/kg/d compared with vehicle-only controls; on d10 these animals had less inflammatory hepatitis/hepatic injury ($p=0.0472$). No differences in bacteremia were observed. Studies of dextran extravasation/vascular permeability and splenic pathology are pending. Benidipine provided a dose-dependent benefit in *A. phagocytophilum*-infected AG129 mice, suggesting that the capacity to reduce innate immune cell activation and possibly vascular permeability could be a useful adjunct to antimicrobial therapy in human anaplasmosis. More analysis is needed to better describe the overall impact of benidipine on the inflammatory response to *A. phagocytophilum*.

P36

Galleria mellonella infection model for studying immune responses to *Bartonella bacilliformis*

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Innate immune responses play important roles in transmission and dissemination of human pathogens and determining the initial outcome of infections; however, specific innate mechanisms are poorly known for *Bartonella*. The purpose of this study was to evaluate an alternative invertebrate model of infection by testing the susceptibility of *Galleria mellonella* larvae (GML) to *Bartonella bacilliformis* (BB) and characterizing the GML responses to infection.

5th instar GML were injected with 10^4 to 10^7 genome equivalent live or heat-killed BB and observed daily for up to 96 hours. Hemolymph and fat body were harvested, and total DNA and RNA were prepared. cDNA was generated and quantified using gene-specific SYBR-Green qPCR. Relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method.

GML inoculated with live BB exhibited dose-dependent melanization, decline of health scores, and death compared to diluent control at either 28 or 34°C. BB was detected microscopically in the hemolymph of infected GML during the first 24 hours of infection, but BB was cleared by 48 hours as determined by PCR. GML developed dose-dependent systemic melanization and died when injected with BB treated at 56°C, 70°C or 95°C. These symptoms were most pronounced with BB heated at 70°C and 95°C, suggesting a thermostable moiety of *Bartonella* modulates early responses of larvae. Transcriptional expression of 3 antimicrobial peptides, prophenoloxidase (PPO), peptidoglycan recognition protein B (PRPB) and apolipophorin-III (ApoLp-III) changed significantly in infected GML compared to control larvae ($P<0.05$). Expression of PPO reached its maximum by 2 hours after infection and then declined through 24 and 48 hours. Expression of ApoLp-III and PRPB genes reached their maximum at 24 hours and declined at 48 hours post infection.

In conclusion, *Galleria mellonella* is susceptible to *Bartonella bacilliformis* and can be used for studying immune responses to *Bartonella*.

P37

Orientia tsutsugamushi Ank13 promotes degradation of mindbomb1 to dysregulate Notch signaling

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Orientia tsutsugamushi is an obligate intracellular bacterium that causes the globally emerging disease, scrub typhus. During infection, *O. tsutsugamushi* expresses a battery of eukaryotic-like ankyrin repeat (AR)-containing effectors (Anks). The majority of these exhibit a bipartite structure with an N-terminal AR domain that facilitates protein-protein interactions and a C-terminal F-box that recruits the host SCF E3 ubiquitin ligase complex to promote proteasomal degradation of interacting partners. Most target proteins and cellular pathways modulated by these Anks are unknown. We recently defined Ank13 as an *O. tsutsugamushi* nucleomodulin that downregulates transcription of numerous genes including those involved in antimicrobial responses. A yeast two-hybrid screen identified mindbomb1 (MIB1) as a putative Ank13 binding partner, which was confirmed by co-immunoprecipitation. MIB1 initiates canonical Notch signaling by activating Notch ligands at the host plasma membrane, ultimately leading to upregulation of Notch-related antimicrobial response and other genes in neighboring cells. *O. tsutsugamushi* reduces MIB1 levels in a bacterial burden-dependent manner. This effect is phenocopied in uninfected cells ectopically expressing Ank13, but not an Ank13 mutant with a functionally inactivated F-box. In *O. tsutsugamushi*-infected cells, Notch ligand surface presentation is upregulated, and Notch-related gene expression is unchanged compared to uninfected cells, indicating that *O. tsutsugamushi* impairs Notch signaling activation upon infection. Notably, host cells ectopically expressing Ank13 exhibit similar alterations in Notch ligand surface presentation and downregulation of Notch-related gene expression. Together, these data indicate that *O. tsutsugamushi* Ank13 binds MIB1 and promotes its proteasomal degradation to impair Notch ligand activation and inhibit transcription of Notch-related genes. Thus, this study provides a novel mechanism by which *O. tsutsugamushi* Ank13 inhibits activation of the Notch signaling pathway to modulate the host antimicrobial response to infection.

P38

Analysis of Ank1 and Ank6 proteins from different *Orientia tsutsugamushi* strains for the ability to inhibit NF-κB

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Orientia tsutsugamushi is a causative agent of the potentially fatal and globally emerging disease, scrub typhus. This obligate intracellular bacterium invades and replicates within leukocytes and endothelial cells to cause disease in humans. As part of its survival strategy in eukaryotes, *O. tsutsugamushi* subverts host immune responses. The mechanisms it uses to do so are poorly understood. Nuclear factor kappa-B (NF-κB), a central activator of innate and adaptive immune responses, translocates to the nucleus and induces proinflammatory gene transcription in response to microbial infections. We previously reported that *O. tsutsugamushi* strain Ikeda prevents NF-κB nuclear accumulation and linked this phenomenon to two of its ankyrin repeat-containing effectors, Ank1 and Ank6. However, the abilities of other Ikeda Anks to inhibit NF-κB nuclear accumulation were not quantitatively assessed. It was also unknown whether the ability to inhibit NF-κB nuclear accumulation is exhibited by Ank1 and Ank6 homologs of other *O. tsutsugamushi* strains or an Ikeda Ank1 paralog (Ank1_01) that lacks most of its ankyrin repeat domain. Here, we validated that Ank1 and Ank6 are the only 2 of the entire cohort of 19 distinguishable Ikeda Anks that robustly impede NF-κB nuclear accumulation when ectopically expressed. *ank1* and/or *ank6* homologs are encoded within 7 of the 8 fully annotated *O. tsutsugamushi* strain genomes. Ectopically expressed Ank1 and Ank6 homologs from strains Karp, TA686, UT76, and Ikeda each inhibit TNFα-stimulated NF-κB nuclear accumulation, while the two truncated Ank1 homologs do not. Thus, Ank1 and Ank6 proteins encoded by numerous *O. tsutsugamushi* strains are capable of negatively regulating NF-κB.

P39

Non-coding RNA mediated regulation of *Orientia* coding transcriptome during host-pathogen interaction

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Scrub typhus, caused by *Orientia tsutsugamushi* (*Ot*), is an underdiagnosed and underreported febrile illness with more than one million cases reported annually. Upon transmission by the *Leptotrombidium* mites, *Ot* rapidly adjusts their virulence and fitness to prevent eradication from the mammalian hosts, yet our knowledge of their adaptation to a host cellular niche conducive to growth and replication remains completely obscure. Bacterial small noncoding RNAs (sRNAs) are now established as important post-transcriptional regulators of adaptation and virulence. Despite their importance, there has been a complete lack of information on the sRNA repertoire and its functions, specifically their regulatory roles in interactions of free, intracytoplasmic *Ot* with the host cells. We have determined the transcriptional landscape of *Ot* strain Karp (*Ot*K) during the infection of human endothelial cells and identified 21 trans-acting (intergenic), 20 cis-acting (antisense), and 13 riboswitches/UTR novel sRNAs (*Ot*K_sR's) employing combinatorial approaches of bioinformatics and RNA sequencing. Notably, while homologs of 19 trans-acting sRNAs were identified in other pathogenic strains of Ot, two sRNAs (OtK_sR21 and 56) were unique to the Karp strain. The expression of novel *Ot*K_sR's was further confirmed in lung and brain tissues of *Ot* infected C3H/HeN mice *in vivo*. Interestingly, all eight *Ot*K_sR's tested were significantly upregulated in the lungs at both 3 and 7 dpi, however, the expression of *Ot*K_sR40 and 57 was only detectable on day 7 in the brain. Further, we have predicted 1169 target genes (p-value of seed region ≤0.05) potentially regulated by 21 trans-acting sRNAs and experimentally validated the interaction of *Ot*K_sR56 with *Karp_00333* (transcriptional regulator) employing heterologous reporter assays. In sum, our studies convincingly demonstrate the existence of post-transcriptional riboregulatory mechanisms in *Ot*. Further studies deciphering the role of these sRNAs in target organ systems in vivo are in progress.

P40

Impairment of host glycolysis fails to inhibit intracellular replication of the nutrient parasite *Orientia tsutsugamushi*

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The obligate intracellular bacterium *Orientia tsutsugamushi* is a causative agent of scrub typhus, a globally emerging disease with an estimated one billion people at risk. Following invasion of host cells, *O. tsutsugamushi* resides within its replicative niche, the eukaryotic cytoplasm, where most of host central metabolism occurs (e.g., glycolysis and the pentose phosphate pathway). *O. tsutsugamushi* is predicted to be metabolically deficient based on genome-derived metabolic reconstructions identifying that the bacterium lacks 50% of the enzymes in each pathway of central metabolism. Thus, the ability of *O. tsutsugamushi* to proliferate within the cytoplasm suggests that the pathogen relies on host-derived metabolites for essential nutrients. Utilizing a combination of targeted and untargeted approaches, experimental evidence revealed that both host metabolites and biosynthetic pathways of central metabolism are modulated during *O. tsutsugamushi* infection. Together, these data implicate host glycolysis as a potential pathway parasitized by *O. tsutsugamushi*. To assess the importance of host glycolysis in *O. tsutsugamushi* infection, we monitored bacterial replication in the presence or absence of a competitive glucose inhibitor, 2-deoxy-D-glucose (2-DG), at discrete time points throughout the infectious cycle. As expected with reduced flux through the first steps of glycolysis, host cells cultured in the presence of 2-DG consumed less overall glucose from the medium and were deficient in replication. Despite this, *O. tsutsugamushi* growth in 2-DG treated host cells was comparable to the untreated condition. Collectively, this study suggests that while host glycolysis is altered during *O. tsutsugamushi* infection, glycolysis in of itself is not required to support intracellular replication, providing novel insights into the metabolic requirements of this bacterial parasite.

P41

The Importance of Epidemiological Surveillance in Sentinel Hosts in the Identification and Monitoring of Areas with a Risk of Re-emergence of Tick-Borne Diseases

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For many tick-borne organisms, both feral and domestic dogs may be utilized as sentinel hosts in order to estimate the risk of human infection. A direct correlation was demonstrated between an increase of seropositivity of these pathogens in the sentinels and the resulting threat upon the public health of humans. Therefore, the prevention and/or control of these zoonotic diseases is contingent upon detection and treatment of the seropositive sentinel animals.

The Central Laboratory of Public Health of Minas Gerais from the Ezequiel Dias Foundation (FUNED) is a National Reference Laboratory for Rickettsiosis, and is a branch of the Brazilian Ministry of Health (BMH). Currently, BMH requires FUNED to use Indirect Immunofluorescence Assays (IFA) in order to provide epidemiological surveillance of *Rickettsia* spp., and *Ehrlichia canis* in sentinels.

Despite *Anaplasma phagocytophilum* being endemic in Brazil, this agent is not currently included in the standard protocol for surveillance by FUNED. In collaboration with Fuller Laboratories, it was proposed that the addition of this agent to their protocol for surveillance could potentially provide benefits to the public health.

Eighty-eight canine sera samples were tested by IFA for the detection of IgG antibodies against *Rickettsia* spp., *E. canis* and *A. phagocytophilum*. From the 88 samples, the following tested positive: 25% for *Rickettsia* spp.; 47% for *E. canis*; 70% for *A. phagocytophilum* and 14% for a co-infection with all three pathogens. Of the samples that tested positive for *A. phagocytophilum*, 39% tested negative for both *Rickettsia* spp. and *E. canis*, and had escaped detection using the current protocol for surveillance.

Epidemiological surveillance of sentinel hosts has direct implications on the prevention and control of zoonotic diseases. Thus, increasing the standard protocol for surveillance to include even more endemic pathogens could prove benefits to the public health.

P42

Field-evaluation of screening tests for the diagnosis of Q fever in breeding bucks and rams.

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Coxiella burnetii is a zoonotic bacterium causing Q fever, a disease that manifests as abortions in small ruminants. Actual surveillance protocols in goat and sheep herds focus on the detection of the bacterium in individual birth products or bulk tank milk. However, considering the venereal potential of Q fever, breeding males may act as excellent sentinels to check the sanitary status of a herd as they mate with many females of the same flock. We conducted a field study to evaluate four types of screening tests to diagnose Q fever in breeding males: PCR on genital swabs, indirect ELISA for *C. burnetii*-specific antibodies in serum, recall assay to detect interferon (IFN) γ in whole blood upon stimulation (24h and 48h) with *C. burnetii* antigens and intradermal testing. Samples (n=934) of all breeding males and at least 20% of the females were collected at regular intervals, before and after the mating season, in three naturally Q fever-infected and five naive small ruminant farms. Using preliminary data, the sensitivity (Se) and specificity (Sp) of the different tests were calculated and compared. In absence of a gold standard test to ascertain the individual Q fever status, all animals of the infected herds were considered positive. The intradermal test was inconclusive as all results were negative and was further excluded from the analysis. When considering equivocal results as positive, the PCR test and ELISA had a Se and Sp of 4% and 100%, and 28% and 95%, respectively. IFNγ tests increased the Se with limited impact on Sp, reaching values of 52% and 94% (24h stimulation), and 59% and 94% (48h stimulation), respectively. In conclusion, the IFNγ test showed a remarkably higher Se than the other tests and could be suggested as a tool to determine the Q fever status of individual animals.

P43

Development of a 23S rRNA real-time reverse transcription PCR assay for detection of *Rickettsia typhi* and *Rickettsia felis* in clinical specimens

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Rickettsia typhi and *R. felis* are etiological agents of flea-borne rickettsioses worldwide, causing murine typhus and flea-borne spotted fever, respectively. Rickettsioses present diagnostic challenges due to low levels of bacteremia and generalized symptoms of rickettsial infections. Serologic detection of IgG is the standard for *Rickettsia* diagnosis. However, serological diagnosis tends to be retrospective due to delay in antibody formation, and IgG cross-reactivity at the species level means IgG testing can only reliably diagnose to spotted fever or typhus group. A real-time reverse transcription PCR (RT-PCR) assay was developed that targets species-specific regions of the 23S rRNA of *R. typhi* and *R. felis*. A single rickettsial bacillus contains multiple copies of 23S rRNA, which allows improved sensitivity over a genomic target. Our 23S rRNA *R. typhi* analyte was 10-fold more sensitive and the *R. felis* analyte was 1000-fold more sensitive than a published duplex *R. typhi* and *R. felis* real-time PCR targeting *ompB*. The assay was assessed against 121 specimens, including 91 clinical specimens collected in California from 2017-2023 and 30 contrived *R. felis* specimens. In clinical specimens, the 23S rRNA assay detected 35 *R. typhi* and 2 *R. felis* samples compared to 32 and 0, respectively, with the *ompB* assay. There were 13 discrepancies noted among the 121 specimens; all but two were resolved using nested RT-PCR and sequencing, showing that the 23S assay correctly detected 11 of the discrepancies. These results show that *R. typhi* is the dominant cause of seropositive flea-borne rickettsioses in California and that the 23S rRNA assay has superior analytical sensitivity, allowing for more accurate diagnosis and molecular confirmation of suspected cases of typhus group rickettsiosis.

P44

Antigenic salivary proteins from *Amblyomma sculptum*: potential targets to control ticks and block *Rickettsia rickettsii* transmission.

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Rocky Mountain spotted fever (RMSF), also known as Brazilian spotted fever (BSF), is caused by the *Rickettsia rickettsii* bacterium, and transmitted by tick saliva. The main vectors in Brazil are *Amblyomma sculptum* and *A. aureolatum*. Immunomodulatory salivary proteins can alter the vertebrate host physiology, assuring tick feeding and also facilitating/enhancing infection of pathogens co-injected with saliva. Since bioactive salivary proteins are important for the establishment of infection, immunization with these proteins can protect the host against the tick and the pathogen transmitted by them.

In order to identify salivary immunogenic proteins, C3H/HePas mice were immunized with *A. sculptum* saliva and an immune serum was produced. In addition, the saliva was submitted to two steps of fractionation by UFLC. The serum of immunized animals presented specific IgG1 and IgG2a antibodies against the salivary proteins in fractions eluted with 58-66% and 50.5-70.5% of ACN in the first and second step of fractionation, respectively. The proteins with humoral reactivity present in fractions from both steps of purification were identified by LC-MS-MS. The genes encoding nine identified proteins were cloned into the VR2001 TOPO plasmid and used for immunizations of C3H/HeN mice, a *R. rickettsii* susceptible strain. Immunizations were performed using pool of plasmids: pool I (1, 2 & 3), II (4, 5 & 6) and III (7, 8 & 9). The empty vector was used as a negative control. Increased IgG1 and IgG2a levels were observed in the serum of mice immunized with pool III and I, respectively. Interestingly, mice immunized with pool I and II showed a significant increase in foot-pad thickness at 6 h and 24 h, respectively, suggesting different profiles of delayed-type hypersensitivity (DTH).

Results revealed proteins with antigenic properties in *A. sculptum* saliva, which can be prospected as potential candidates to develop vaccines for tick control and/or blocking of *R. rickettsii* transmission.

P45

Environmental drivers of immature Ixodes scapularis in Minnesota's metro area

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Research on the public health significance of *Ixodes scapularis* ticks in the Midwest seldom focuses on abiotic conditions that can modulate their population dynamics and ability to transmit pathogenic organisms. In this study, we assessed whether the distributional abundance of *I. scapularis* immatures is associated with current and time-lagged climatic determinants either directly or indirectly. We analyzed a 20-year longitudinal small mammal live-trapping dataset within a seven-county metropolitan area in Minnesota (1998-2016), estimated yearly tick counts at each site, and asked whether inter- and intra-annual variation in immature *I. scapularis* counts are associated with six climatic variables selected a priori. We found that (1) immature *I. scapularis* ticks infesting mammals expanded south-westerly over the study period, (2) eastern chipmunks supplied a substantial proportion of nymphal blood meals, (3) a suite of climatological variables are demonstrably associated with blacklegged tick presence, and abundance across sites, most notably summer vapor pressure deficit, and (4) immature blacklegged ticks display an affinity for forested habitats in metro areas. Our results suggest that climatic and land-type conditions may impact host-seeking *I. scapularis* ticks through numerous mechanistic avenues. These findings extend our understanding of the abiotic factors supporting blacklegged tick populations in metro areas of the upper Midwest with strong implications for discerning future tick-borne pathogen risk.

P46

Microbiome and nanopore adaptive sampling surveillance for tick-borne pathogens

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PCR-based pathogen detection methods are inherently limited because they must target particular pathogen genetic sequences. This study examined two detection methods leveraged for pathogen agnostic surveillance in ticks: 16S rDNA microbiome sequencing and nanopore adaptive sampling. 355 adult *Ixodes scapularis* were collected from 16 sites from the Upper Midwest and the V4 variable region of the 16S gene was sequenced to determine genus-level microbiome constituents. Where applicable, pathogen species were tested by PCR to determine species-level identifications. A subset of individual ticks co-infected with multiple pathogenic bacteria were evaluated using nanopore sequencing and the nanopore adaptive sampling bioinformatic pipeline tuned to enrich DNA sequences from *I. scapularis*-associated pathogens. This method produced long sequences across the pathogen genomes and differentiated closely related pathogen taxa. These developing nanopore sequencing methods have the potential to greatly enhance data generation in the realm of tick-borne pathogen surveillance and discovery.

P47

Characterization of a novel Anaplasma bovis-like human pathogen in the United States

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Bacteria of the genus *Anaplasma* are obligate intracellular pathogens of both animals and humans throughout the world. Currently, three species are known to cause disease in humans: *Anaplasma phagocytophilum, A. ovis*, and *A. bovis*. During a recent large-scale evaluation of patients with suspected tick-borne disease in the United States by using targeted metagenomics, blood samples from four patients contained DNA of a previously unknown bacteria. Although the study design precluded collection of clinical data, it was determined that the patients resided in Minnesota, Oklahoma, and Missouri, and that the specimens were collected during 2015-2017. Initial analysis of the V1-V2 region of the 16S gene revealed this pathogen to be closely related to *A. bovis*. Additional molecular characterization of this *A. bovis*-like agent using a second region of the 16S gene as well as regions of the *glt*A and *gro*EL genes confirmed the phylogenetic grouping of this bacteria as closely related to *A. bovis*, but also revealed clear genetic differences between this agent and reported *A. bovis* sequences from around the world. Subsequently, 5 *D. variabilis* ticks collected in Oklahoma, Iowa, and Nebraska were also found to contain the DNA of this agent, while a *D. andersoni* tick from Canada contained nearly identical sequence data, to suggest that *D. variabilis* could be a vector of this novel tick-borne human pathogen. Additional work is ongoing to determine the frequency of this novel bacteria in *D. variabilis*, to obtain an *in vitro* isolate to aid in its further characterization and formal species designation, and to develop a specific assay to identify additional patients and better characterize the clinical features and epidemiology of this recently recognized tickborne infection.

P48

High-throughput identification of *Ehrlichia chaffeensis* immunodominant proteins

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Recently, our laboratory implemented a high-throughput antigen discovery approach that includes gene synthesis, cell-free protein expression and rapid immunoscreening to identify previously unknown *E. chaffeensis* immunoreactive proteins. In this study, we completed screening of the entire proteome which included *E. chaffeensis* proteins (n=445) that were not investigated previously. Of these, 197 proteins exhibited some level of reactivity with antibodies in pooled convalescent sera from HME patients. Seven proteins were determined to be immunodominant by strong reactivity with HME sera, comparable to levels observed with well-defined TRP120. The new *E. chaffeensis* immunodominant proteins identified were determined to have conformation-dependent antibody epitopes. Many of these proteins were classified with unknown function and were small (< 25 kDa) in size. The majority of new immunodominant proteins were predicted to be type I secreted effectors yet some contained predicted transmembrane domains. Characterization of complete *E. chaffeensis* immunome will significantly advance our understanding of host-*Ehrlichia* immune responses and identify antigen vital to vaccine development.

P49

Multistrain A. marginale infections are unexpectedly common in a Kansas cattle herd

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Control of bovine anaplasmosis, caused by Anaplasma marginale, presents an increasingly difficult problem for producers. Though the reduction in the use of antibiotics limits the tools available, other less well-understood factors may also play a role. One feature of A. marginale is genetic diversity which allows individual strains to establish long term persistent infection and overcome existing immunity in an infected host to establish superinfection. These multistrain infections lead to within host competition which can shift pathogen traits including transmissibility and virulence. In tropical and subtropical regions where A. marginale prevalence is 95% to 100%, multistrain infections are common and most animals harbor up to 6 strains. In contrast, in temperate regions prevalence tends to be lower, and multistrain infections are thought to be uncommon. However, many knowledge gaps remain regarding the prevalence of multistrain infection in temperate regions. To start to address this knowledge gap, we genotyped seven A. marginale infected animals from a herd in Kansas suffering from high disease incidence and mortality due to bovine anaplasmosis. The strain composition was determined using Msp1a. Among these animals, there were 16 genotypes. Unexpectedly, all animals harbored multistrain infection with 2 to 6 genotypes per animal (median = 5). In contrast in one study, there were 11 strains identified in one herd of 75 animals. Four percent of those animals harbored 2 to 3 three strains, while the remainder had single strain infections. The factors that lead to the unexpectedly high prevalence of multistrain infections in the herd reported here are unknown and may be due to high tick burdens, management practices, and co-infections with other pathogens. Additional studies are required to measure the prevalence of multistrain infections in temperate regions and understand the implications of those infections for the epidemiology and control of bovine anaplasmosis.

P50

Antibodies contribute to vaccine protection against rickettsiae in mice

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We previously demonstrated that a single dose immunization of *Rickettsia parkeri* mutant 3A2 confers complete protection against two fatal rickettsioses in mice. *R. parkeri* 3A2 was generated by inserting a modified pLoxHimar transposon into the gene encoding a phage integrase protein, which significantly attenuated virulence *in vivo*. In the present study, we investigated the mechanisms involved in protection conferred by this potential live-attenuated vaccine (LAV) candidate against rickettsioses. Immunization of C3H/HeN wild-type (WT) mice with *R. parkeri* 3A2 triggered a significantly elevated IgG antibody titer against *R. parkeri* that lasted for five months without decline. *R. parkeri* 3A2-induced IgG antibodies also reacted with *R. conorii*, although at a lower level compared to *R. parkeri*. To determine the role of elevated IgG antibody in vaccine-conferred protection, T- and B-cell-deficient C3H-scid mice were challenged with a lethal dose of *R. parkeri* after passive transfer of diluted serum from mice immunized with 3A2 or control. All infected C3H-scid mice transferred with 3A2-immune serum survived, while all control mice succumbed to infection. C3H-scid mice, intradermally inoculated with *R. parkeri* 3A2 at a dose that is safe and confers protection against fatal rickettsioses in WT C3H mice, died on day 30 post immunization. Taken together, *R. parkeri* 3A2 represents a promising LAV candidate only for immunocompetent individuals. LAV candidate induced rickettsiae-specific antibodies that confer protection against fatal rickettsiosis in mice.

P51

Molecular detection of *Orientia tsutsugamushi* DNA by PCR and qPCR targeting repeat sequences

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Scrub typhus (ST) is a highly neglected, vector-borne disease caused by an obligate intracellular bacteria *Orientia tsutsugamushi* (OT). Many studies indicated a high burden of scrub typhus in India. Diagnosis of ST is quite challenging as disease shares its symptoms with other acute febrile illnesses. Several serological and molecular tests are currently in use for the diagnosis. Serological tests like ELISA, IFA and PCR targeting the *56kDa*, *47kDa* and *groEL* genes are routinely used to detect *Orientia tsutsugamushi*. However, these targets present in one copy in the genome which makes it difficult to diagnose pathogens such as in the early stage of infection. Comparative genomic analysis of 9 complete genomes of OT revealed a presence of a conserved region of repetitive sequences. These sequences are present in multi-copy numbers in different strains of OT genomes. In this work, novel PCR primers along with the probe (for qPCR) were designed to target these regions with amplicon size 154 bp and adapted for the diagnosis of ST by qPCR. First of all, a total n=1378 blood samples were tested to detect O. tsutsugamushi IgM antibody using IgM ELISA. Further, DNA was extracted from the same samples and PCR was done targeting the sequences mentioned above. Out of total n=1378 samples, n=259 (19 %) samples were found positive and 18 were equivocal by IgM ELISA whereas n=336 samples (25%) were found positive by PCR. PCR allows diagnosis of ST even in early infection (fever with 2 days) 11 out of 34 samples, while 2 were detected by ELISA. PCR also detected 211 samples which were negative by ELISA. In conclusion, these novel targets served the purpose of effective and specific diagnosis of OT in the early stages of infection and can be used regularly for monitoring and surveillance purposes.

P52

A genetically modified live vaccine induces long-lasting protection against wild-type Ehrlichia chaffeensis infection by mechanical and tick transmission.

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Ehrlichia chaffeensis is an obligate intracellular tick-borne bacterium causing monocytic ehrlichiosis in people and dogs. We previously reported that the immunization of dogs with a functional disruption mutant of E. chaffeensis gene (ECH_0660) encoding for the phage head to tail connector protein (phtcp) as a modified live vaccine (MLV) induces protection against intravenous and tick transmission challenges four weeks following vaccination. In this study, we evaluated the duration of immune protection by the MLV up to one year. Three groups of dogs were vaccinated with the live vaccine and challenged with wild-type E. chaffeensis after 4, 8 and 12 months by intravenous and tick transmission challenges. All animals were monitored for 28-35 days after the infection challenge. The MLV group animals receiving wild-type infection cleared rapidly irrespective of the transmission routes, as evidenced by the lack of detectable bacteria by molecular assessment. Contrary to this, non-vaccinated infection controls remained positive for the infection all through the assessment period. Immunization with the MLV induced E. chaffeensis-specific T cell responses detectable by ELISPOT for IFNγ and differentiation of IFNγ- and/or TNFα- producing pathogen-specific T effector memory CD4 T cells (CD45RAnegCD45RO+CD62Lneg). Antigen-specific recall responses were also detectable in the peripheral blood of most vaccinated dogs. This is the first study demonstrating the efficacy of phtcp mutant live vaccine in protecting the canine host for one year against E. chaffeensis infection by mechanical and tick transmission routes.

P53

Recombinant Adenovirus Expressing P56 Antigen as Vaccine Candidates for Protection against Scrub Typhus Infection

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Orientia tsutsugamushi is the etiologic agent of scrub typhus, which is the most prevalent rickettsiosis worldwide. *Orientia* is maintained transovarially by its reservoir hosts, trombiculid mites, which emerge as larvae from the soil seeking a meal. Humans develop an eschar at the site of inoculation, and subsequently the bacteria disseminate hematogenously throughout the body with infection of endothelial cells and macrophages. This study aims to develop and evaluate Orientia P56 antigen-expressing recombinant non-replicative adenovirus vectors for protection against scrub typhus infection with *O. tsutsugamushi* in mice. P56 (TSA56) has been found to stimulate protective immunity against lethal challenge of *O. tsutsugamush*i. Replication-deficient recombinant human adenovirus 5 constructs containing the entire ORF or the four hypervariable domains of P56 *O. tsutsugamushi* Karp strain were prepared for mice immunizations after confirmation of recombinant protein expression by Western blot analysis. Mice were injected intramuscularly with 1 x 10^7 IFU, followed by a booster immunization three weeks later, and challenge by tail vein inoculation three weeks after the booster immunization with 3 LD_{50} of *O. tsutsugamushi* Karp strain. Mice will be observed for signs of illness (morbidity, temperature, weight loss, inactivity, hunch back posture). Blood will also be collected prior to boost and challenge and assayed for neutralizing antibodies. This innovative project within the field of scrub typhus research utilizes an assay for neutralizing antibodies, immunization with conformationally accurate antigens in a recombinant adenovirus vector, and comparison of the immune responses stimulated by vaccination versus infection.

P55

TNFα secretion is blocked in *C. burnetii*-infected bovine macrophages

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Coxiella burnetii, the causative agent of Q fever, is a zoonotic pathogen, infecting humans, livestock, pets, birds, and ticks. Domestic ruminants such as cattle, sheep, and goats are the main reservoir. Infected ruminants are usually asymptomatic, but reproductive disorders might occur. In addition, infected ruminants are the major cause of human infection, which mainly occurs via inhalation of contaminated aerosols. In humans, the infection might result in acute Q fever, which manifests as a flu-like illness, hepatitis, or pneumonia. In very rare cases, the infection can become chronic with a potentially lethal endocarditis as the most common manifestation. Macrophages are the main target cells of *C. burnetii*. Here we analyzed the interaction of *C. burnetii* with primary human and bovine macrophages and confirmed that human macrophages prevent *C. burnetii* replication under oxygen-limiting (hypoxic) conditions. In contrast, oxygen content had no influence on *C. burnetii* replication in bovine macrophages. In hypoxic infected bovine macrophages, STAT3 is activated, even though HIF1α is stabilized, which otherwise prevents STAT3 activation in human macrophages. In addition, the TNFα mRNA level is higher in hypoxic than normoxic human macrophages, which correlates with increased secretion of TNFα and control of *C. burnetii* replication. In contrast, oxygen limitation doesn´t impact TNFα mRNA levels in *C. burnetii*-infected bovine macrophages and secretion of TNFα is blocked. As TNFα is also involved in the control of *C. burnetii* replication in bovine macrophages, this cytokine is important for cell autonomous control and its absence is partially responsible for the ability of *C. burnetii* to replicate in hypoxic bovine macrophages. Further unveiling the molecular basis of macrophage-mediated control of *C. burnetii* replication might be the first step towards the development of host directed intervention measures to mitigate the health burden of this zoonotic agent.

P56

Egress mechanisms of *Coxiella burnetii*

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Coxiella burnetii is a Gram-negative, obligate intracellular pathogen and the causative agent of Q fever. The clinical presentation of the acute infection can vary from self-limiting flu-like illness to an atypical pneumonia or hepatitis. In rare cases chronic Q fever develops, with endocarditis as the most common manifestation. Importantly, chronic Q fever is characterized by a high mortality rate, especially if treatment is delayed. Resident alveolar macrophages are the first target cells, but *C. burnetii* spread to other organs and cell types including endothelial cells. While we have information about the initial step of *C. burnetii* uptake and the maturation process of the *Coxiella*-containing vacuole, it is completely unknown how *Coxiella* spreads, i.e. how it exits its host cell to enter new target cells. Understanding this essential step during infection will be crucial to develop novel strategies to prevent *C. burnetii* spreading and, thereby, disease.

Here we demonstrate that *C. burnetii* is released from infected cells at late stages of infection. Substantially, prior establishment of a large bacteria-filled vacuole seems to be a prerequisite for egress. Released bacteria can infect new target cells even without cell-cell-contact and are capable of replication. The egressed *Coxiella* are susceptible to gentamicin, suggesting that they are not covered by a host cell membrane. Electron microscopy images support this assumption. How the release of *C. burnetii* is induced and regulated is still an open question. Our preliminary data suggest that induction of host cell apoptosis is essential for egress, as we observed a significant reduction from cells with an impaired intrinsic apoptotic signaling cascade. Importantly, cells infected with a defective type IV secretion system (T4SS) mutant do not allow spreading of *C. burnetii*. In summary, our data argues for *C. burnetii* egress in a non-synchronous way by host cell apoptosis-induction, possibly via the T4SS.

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The *Chlamydia trachomatis* secreted effector protein CT181 binds to the apoptosis regulator, Mcl-1, to promote infection

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Chlamydia trachomatis is an obligate intracellular pathogen. Formation of its unique replicative niche, termed the inclusion, requires an arsenal of secreted effector proteins to be released into the host cell. A subset of these proteins have been implicated in host cell invasion and subversion of host defense mechanisms, allowing chlamydia to initiate infection. While secreted effectors including TmeA, TarP, TmeB, and TepP have risen to prominence as key players in this process, additional factors are likely secreted to promote chlamydia infection. Here we used three specialized secretion assays to determine whether additional hypothetical proteins that are uniquely produced by the infectious elementary body represent secreted factors. These combined approaches identified 7 effectors that were released into the host cell cytosol during chlamydial infection, 3 of which had not been previously predicted to be secreted. Site-specific mutagenesis using the TargeTron revealed that one of these, CT181, is important for intracellular replication and inclusion development, but is dispensable for host cell invasion. Yeast-2-hybrids and affinity purification mass spectrometry revealed that CT181 binds to the induced myeloid leukemia cell differentiation protein 1 (Mcl-1). Mcl-1 has a diverse set of roles in the cell including apoptosis regulation, which has previously been shown to be important for chlamydial infection and is likely why CT181 modulates it. In support of this observation, we demonstrate that CT181 and Mcl-1 localize to the mitochondria. Collectively our data suggests that CT181 might target Mcl-1 to modulate apoptosis during the initial stages of chlamydia infection.

P58

Characterizing strain-specific host-pathogen interactions that modulate *Coxiella burnetii* infection

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Coxellia burnetii is a gram-negative intracellular bacterium that causes the human disease Q fever, which can present as an acute flu-like illness or persistent, focalized infection. Mammals, including livestock, are a natural reservoir for *C. burnetii* and most human infections are acquired by inhalation of contaminated aerosols derived from these animals. In 1957, at the Dugway Proving Grounds, Utah, USA, genetically unique strains of C. burnetii were isolated from rodents. Despite the presence of full-length, phase I lipopolysaccharide (LPS), Dugway strains have been reported to exhibit attenuation in vivo. In addition to LPS, the C. burnetii Dot/Icm type 4 secretion system (T4SS) is an important virulence determinant that delivers effector proteins required for the development of the *Coxiella*-containing vacuole and bacterial replication. Genome sequencing has revealed numerous genes encoding putative effector proteins that are unique to Dugway. These genes were assayed for bacterial secretion using an adenylate cyclase reporter assay and 13 out of 61 putative effectors tested were actively secreted. To further characterize the confirmed secreted effector proteins, fluorescently tagged Dugway effector proteins are currently being tested in mammalian ectopic expression assays. Preliminary results have demonstrated differences in effector localization patterns suggesting, in some cases, specific host-effector interactions. RNA sequencing of human monocytic cells infected with either virulent nine mile I (NMI) or Dugway showed that Dugway-infected cells had distinct gene expression patterns compared to cells infected with NMI, particularly in genes associated with innate inflammatory responses. Additionally, to compare differences in bacterial gene expression during infection, *C. burnetii* transcriptomes of both NMI and Dugway are currently being analyzed. Together, this research will provide important information about the specific mechanisms and host-pathogen interactions that promote *C. burnetii* virulence.

P59

Host Lipid Transport Protein ORP1 Is Necessary for *Coxiella burnetii* Growth and Vacuole Expansion in Macrophages

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Coxiella burnetii is an intracellular bacterium that causes the human disease Q fever. *C. burnetii* forms a large, acidic *Coxiella*-containing vacuole (CCV) and uses a type 4B secretion system to secrete effector proteins into the host cell cytoplasm. While the CCV membrane is rich in sterols, cholesterol accumulation in the CCV is bacteriolytic, suggesting that *C. burnetii* regulation of lipid transport and metabolism is critical for successful infection. The mammalian lipid transport protein ORP1L (oxysterol binding protein-like protein 1 Long) localizes to the CCV membrane and mediates CCV-endoplasmic reticulum (ER) membrane contact sites. ORP1L functions in lipid sensing and transport, including cholesterol efflux from late endosomes and lysosomes (LELs), and the ER. Its sister isoform, ORP1S (oxysterol binding protein-like protein 1 Short) also binds cholesterol but has cytoplasmic and nuclear localization. In ORP1-null cells, we found that CCVs were smaller than in wild-type cells, highlighting the importance of ORP1 in CCV development. This effect was consistent between HeLa cells and murine alveolar macrophages (MH-S cells). CCVs in ORP1-null cells had higher cholesterol content than CCVs in wild-type cells at 4 days of infection, suggesting ORP1 functions in cholesterol efflux from the CCV. While the absence of ORP1 led to a *C. burnetii* growth defect in MH-S cells, there was no growth defect in HeLa cells. Together, our data demonstrated that *C. burnetii* uses the host sterol transport protein ORP1 to promote CCV development, potentially by using ORP1 to facilitate cholesterol efflux from the CCV to diminish the bacteriolytic effects of cholesterol.

P60

Mechanisms of Coxiella burnetii Infection Induced Autoantibodies in Mice

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Coxiella burnetii is an obligate intracellular bacterium that causes acute and chronic Q fever in humans. Autoantibodies have been detected in acute and chronic Q fever patients. Clinical case reports suggest that C. burnetii infection can cause autoimmune hepatitis and reactive arthritis. However, the mechanism of C. burnetii infection induced autoimmunity remains unclear. To determine if C. burnetii infection can induce autoimmunity in mice, immunoblotting with host cell antigens was used to detect autoantibodies in C. burnetii infected mouse sera. The observation that immune sera bound to several different size antigens from both mouse and human cell lines suggests that C. burnetii infection can induce autoantibody response in mice. ELISA analysis indicated that the autoantibodies to nuclear, mitochondrea, dsDNA and ssDNA were detectable in mouse sera from C. burnetii infected mice. This is the first evidence to demonstrate that C. burnetii infection can induce autoantibodies in mice. Our concurrent study indicates that MyD88 deficiency in mice significantly decreased inflammatory response to C. burnetii infection in liver and spleen, suggesting MyD88 plays a critical role in C. burnetii infection induced inflammatory response. To investigate if MyD88 is involved in C. burnetii infection induced autoimmunity, the autoantibodies to nuclear, mitochondrea, dsDNA and ssDNA were examined in mouse sera from C. burnetii infected MyD88 deficient mice at different time points post-infection. Interestingly, compared to WT mice, autoantibodies significantly decreased in MyD88 deficient mice in response to C. burnetii infection. In addition, isotype analysis of autoantibodies demonstrated that IgM and IgG3 were the major isotypes of autoantibodies and that IgM and IgG3 subclass autoantibody responses significantly decreased in C. burnetii infected MyD88 deficient mice. These results suggest that Toll-like receptor-mediated MyD88 dependent signaling pathway may play an important role in C. burnetii infection induced autoimmunity.

P61

STING-dependent BAX-IRF3 signaling results in apoptosis during late-stage Coxiella burnetii infection

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STING (STimulator of Interferon Genes) is a cytosolic sensor for cyclic dinucleotides (CDNs) and initiates an innate immune response upon binding to CDNs. Coxiella burnetii is a Gram-negative obligate intracellular bacterium and the causative agent of the zoonotic disease Q fever. The ability of C. burnetii to inhibit host cell death is a critical factor in disease development. Previous studies have shown that C. burnetii inhibits host cell apoptosis at early stages of infection. However, by late stages of infection, there is host cell lysis resulting in the release of bacteria to infect bystander cells. Thus, we investigated the role of STING during late stages of C. burnetii infection and examined STING's impact on host cell death. We show that the loss of STING results in higher bacterial loads and abrogates IFNβ and IL6 induction at 12 days post-infection. The absence of STING during C. burnetii infection significantly reduces apoptosis through decreased caspase-8 and -3 activation. During infection, STING activates IRF3 which interacts with BAX. BAX then translocates to the mitochondria, which is followed by mitochondrial membrane depolarization. This results in increased cytosolic mtDNA in a STING-dependent manner. The presence of increased cytosolic mtDNA resulted in greater cytosolic 2'-3' cGAMP leading to positive feedback to STING activation and its downstream signaling. Taken together, we show that STING signaling is critical for BAX-IRF3-mediated mitochondria-induced apoptosis during late-stage C. burnetii infection.

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Non-specific Immune Induction via Distinct Routes of Vaccination and Infection with *Coxiella burnetii*

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Coxiella burnetii is a Gram-negative, obligate intracellular pathogen and the causative agent of the human disease, Q fever. *C. burnetii* whole cell vaccination (WCV) induces potent, long-term protection against Q fever; however, there limited data indicating that beyond *C. burnetii*-specific protection, WCV may elicit non-specific immune (NSI) protection against distinct pathogens. Many important questions surrounding NSI induction in the context of both *C. burnetii* vaccination and infection remain unanswered. Here, we investigated *C. burnetii* NSI induction using mouse models of infection and vaccination. We performed *ex vivo* stimulatory assays on bone marrow derived macrophages (BMDMs) isolated from mice vaccinated or infected with *C. burnetii* by distinct routes. Further, we utilized virulent (NMI) and attenuated (NMII) strains of *C. burnetii* and collected bone marrow at fourteen days post vaccination or infection. We observed alterations in the production of proinflammatory cytokines, TNFa and IL-6, in BMDMs isolated from mice vaccinated or infected *C. burnetii* post LPS and *C. albicans* stimulation ex vivo when compared to mock treated animals. Compared to intranasal (IN) vaccination, subcutaneously (SC) vaccinated animals exhibited more pronounced changes in post-stimulation BMDM cytokine production. BMDMs of IN infected mice exhibited similar changes to those of SC vaccinated post LPS stimulation but displayed more pronounced responses when stimulated with *C. albicans* than those of SC vaccinated mice. These NSI-related changes were dependent on the strain of *C. burnetii* used. Similar experiments performed in primary human monocytes revealed increased NSI-related cytokine responsiveness when compared to mice. While mice are not the optimal small animal model for Q fever, these small, but quantifiable alterations in NSI-related cytokine induction, suggest that *C. burnetii* induces NSI. This data will broaden our knowledge of NSI, and ultimately, contribute to the development of improved vaccines for Q fever and beyond.

P63

Coxiella burnetii Alters Human Macrophage Metabolism and Mitochondrial Physiology

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Coxiella burnetii, the etiologic agent of Q fever, is a Gram-negative intracellular bacterium that infects humans via inhalation of contaminated aerosols. *C. burnetii* initially targets alveolar macrophages to establish an intracellular replication niche within a phagolysosome-like compartment termed the *Coxiella*-Containing Vacuole (CCV). *C. burnetii* encodes a type IV secretion system (T4SS), which is deployed to secrete effector proteins that coerce host cell functions to benefit the bacterium, orchestrating an immunosuppressive pro-bacterial environment wherein the pathogen replicates to high numbers. However, the impact of host metabolic function on establishment of favorable intracellular conditions is undefined. Using Seahorse extracellular flux analysis, we show that *C. burnetii* maintains host oxidative phosphorylation (OXPHOS) at homeostasis in a T4SS-dependent manner. Inhibiting OXPHOS results in deficient CCV expansion, while glycolysis and fatty acid oxidation inhibition do not alter vacuole formation. Interestingly, increased mitochondrial fission occurs in infected cells, indicating *C. burnetii* manipulation of mitochondria, potentially to influence host metabolism. Finally, endoplasmic reticulum (ER) stress regulates immunosuppressive phenotypes in macrophages, and *C. burnetii* regulates ER stress in a T4SS-dependent manner. Here, we show involvement of the ER stress-related kinase PERK in regulating OXPHOS during infection. Collectively, our results indicate that *C. burnetii* engages human macrophage metabolic processes to establish a replication niche.

P64

Identification of long non-coding RNAs lnc-CYP1B1 and lnc-DKK2 as potential biomarkers for *Coxiella burnetii* infection

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Coxiella burnetii (Cb) is an obligate intracellular respiratory pathogen that grows within host monocytes and macrophages and causes Q fever. Cb replicates inside the Coxiella containing vacuole (CCV) and promotes its intracellular growth by modulation of immune responses through poorly characterized mechanisms. Eukaryotic long non-coding RNAs (lncRNAs) are a regulatory class of transcripts that have emerged as essential inflammation regulators and remain largely uncharacterized especially in regulation of host immune responses during Cb infection. In this study, we adopted a comparative infection model screening approach to identify unique host lncRNAs signatures to Cb infection. CYP1B1-AS1 (lnc-CYP1B1) and lnc-DKK2 were identified as potential diagnostic biomarkers through receiver operating characteristic (ROC) analysis. We found 1692 genes were significantly altered, out of which 161 were differentially expressed (DEG) lncRNAs. The functions of DEG lncRNAs were mainly enriched in regulation of metabolism, endocytic trafficking, regulation of immunity effector and receptor response. lnc-DKK2 and lnc-CYP1B1 enriched for Wnt signaling, JAK-STAT, TNF-α and ROS meditated signaling response. Our in-vivo results showed these lncRNAs exhibited spatio-temporal specific expression across 14 days of infection with NMI. Coding potential assessment analysis showed these annotated lncRNAs were non-coding in nature. Further, polyadenylation and cellular localization experiments showed lnc-DKK2 and lnc-CYP1B1 were nuclear enriched polyadenylated lncRNAs. Mechanistically, lnc-CYP1B1 shares bidirectional promoter with CYP1B1, lnc-DKK2 and DKK2 have divergent promoters and they regulate gene expression in cis. Collectively, these findings unveil the role of novel lncRNAs in modulation of host signaling pathways which will be further explored for their role in Cb pathogenesis.

P65

The Contribution of *C. burnetii* Developmental Stage to Intracellular Pathogenesis

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Coxiella burnetii is a Gram-negative, obligate, intracellular bacterium and the causative agent of the zoonosis Q fever, a highly infectious respiratory disease. This bacterium has a biphasic cell morphology. The small cell variant (SCV) is the inert, extracellular form. This form is believed to be the cause of natural infection due to its environmental stability. The large cell variant (LCV) is intracellular and fragile. During infection, *Coxiella* is inhaled into the lungs and phagocytosed by alveolar macrophages, where it is trafficked to a pathogen-tailored compartment called the *Coxiella* containing vacuole (CCV), which resembles a terminal lysosome. While in this CCV, the SCV transitions into an LCV and begins to replicate. Both forms have been described as infectious using *in vitro* models of infection in immortalized cell lines. However, these cellular infection models are largely irrelevant to pathogenesis *in vivo* due to traditional cell lines used. We have developed a primary macrophage infection model that better replicates the effects of Q fever at the cellular level. These bone marrow derived macrophages (BMDM) retain the bactericidal activity that is not conserved in traditional cell lines. We hypothesize that the SCV's extraordinary environmental stability imparts an enhanced ability to survive the BMDM phagolysosome relative to the LCV form. Our plan to test this hypothesis is to separate the SCV and LCV populations and test their ability to infect and replicate in BMDMs. We will generate pure populations of each cell type through density gradient centrifugation. We will also move to mouse models after the *in vitro* testing. By resolving the infectious potential of these two cell types, we will better understand Q fever infection dynamics and disease progression.

P66

Development of a Lipid-based Method for Fluorescent Labeling of *Coxiella burnetii*: A Work in Progress

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As the etiologic agent of Q fever, *Coxiella burnetii* is a Gram-negative bacterium with significant scientific and clinical relevance. Currently, Q-Vax is the only commercially available preventative vaccine, however due to severe local and systemic reactions, it is only approved in Australia. These adverse reactions are collectively referred to as post-vaccination hypersensitivity (PVH) responses and are a form of granulatomous type IV delayed hypersensitivity resulting in leukocyte localization and subsequent severe inflammation and abscess formation. Although the PVH response is somewhat understood from the host perspective, the mechanisms of vaccine and pathogen dissemination are poorly understood, which in turn, contributes to the elusivity of reaching a full understanding of mechanisms underlying vaccine efficacy and post-vaccination hypersensitivity reactions.

Given the lack of knowledge regarding both infection and vaccination dissemination kinetics as well as the difficulties associated with tracking the bacteria within host tissues, we are developing a method for the labeling of *C. burnetii* with fluorescent lipid dyes for use in future dissemination studies. Labeled *C. burnetii* exhibits detectable levels of fluorescence that appear to be long lasting and are unaffected by fixation methods. Completion of this project should allow for the tracking and visualization of *C. burnetii* dissemination within in vivo animal models for the first time in an easy and efficient manner. Insights into dissemination patterns will be essential to better understand Q fever pathogenesis and inform therapeutic and countermeasure development, especially with regards to the development of an improved vaccine candidate with decreased risk for severe PVH reactions.

P67

Defining the contribution of *dacC* to Q fever pathogenesis

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Coxiella burnetii is an obligate intracellular bacterium that causes the zoonosis Q fever. In humans, it presents as a severe respiratory illness that is usually self-limiting, but rarely causes chronic endocarditis. In domestic ruminants, infection usually results in spontaneous abortions. The bacterium exists in two forms: the small cell variant (SCV) and large cell variant (LCV). The SCV is the extracellular form found in the environment, and is resistant to environmental stress, but incapable of replication. The LCV is intracellular, metabolically active and replicates in human alveolar macrophages. Virulence factors that have been identified to enable *C. burnetii* to replicate in macrophages are few. Specific adaptations of *C. burnetii* to resist macrophages are unknown. The goal of our research is to identify novel virulence factors that are relevant to infection of macrophages. We have identified a gene (*dacC*) that is required for replication only in macrophages. *dacC* is a gene that encodes for penicillin-binding protein 6, a d-d carboxypeptidase involved in bacterial cell wall maturation. We hypothesize that cell wall mutants of *C. burnetii*, like our *dacC* transposon mutant, do not survive in macrophages, as disruption of the cell wall integrity makes it vulnerable to host cell defense mechanisms. Interestingly, we have data to suggest that the *dacC* mutant fails to complete the transition from LCV to SCV. We hypothesize that this aberrant SCV formation may be related to the mutant's inability to resist the environment within macrophages. We are currently investigating this mutant's ability to survive in macrophages and mice. This is the first study to link cell wall structure with *Coxiella* pathogenesis.

P68

Coxiella burnetii manipulates host MiT-TFE proteins to influence lysosome biogenesis and promote infection

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Coxiella burnetii is a highly infectious, gram-negative obligate intracellular bacterium and the causative agent of human Q fever. The *Coxiella* Containing Vacuole (CCV) is a modified phagolysosome that forms through fusion with host endosomes and lysosomes. While an initial acidic pH<4.7 is essential to activate *Coxiella* metabolic activity, the mature, growth-permissive CCV is pH ~5.2 and stable throughout infection. Further acidifying the CCV to a lysosomal pH (~4.7) causes *Coxiella* degradation, suggesting that regulation of CCV pH is important for *Coxiella* to maintain an infection. We recently demonstrated that *Coxiella* inhibits host cell lysosome biogenesis by blocking endosomal maturation, leading to a significant decrease in host lysosomes in infected cells. Lysosome biogenesis is primarily controlled by the host cell Transcription Factor EB (TFEB) which binds CLEAR (coordinated lysosomal expression and regulation) motifs upstream of genes involved in lysosomal biogenesis. TFEB is a member of the MiT/TFE protein family, which is defined by a basic helix-loop-helix (bHLH) structure and also includes MITF, TFE3, and TFEC. To understand the role of the MiT/TFE3 family during *Coxiella* infection, we examined bacterial growth and CCV size in knockout cells. We found that in cells lacking TFEB, both *Coxiella* growth and CCV size increase. Conversely, TFEB overexpression leads to significantly less bacterial growth and smaller CCVs. Together, these data indicate that host cell TFEB restricts *Coxiella* infection. Intriguingly, TFE3, while structurally similar to TFEB, appears to promote *Coxiella* infection based on decreased growth and CCV size in TFE3KO cells. Using luciferase and cellular fractionation assays, we discovered that *Coxiella* actively blocks TFEB nuclear translocation in a Type IV Secretion System-dependent manner, thus decreasing lysosomal biogenesis. Together, these results suggest that to avoid further CCV acidification through heterotypic fusion with host cell lysosomes, *Coxiella* inhibits TFEB nuclear translocation to limit lysosomal biogenesis.

P69

Coxiella burnetii modulates the host cell cycle to establish and mature the CCV

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Modulation of the host cell cycle is a common strategy employed by pathogens to optimize their host as a permissive replicative niche. Our data on secreted effector protein CBU0388 suggests Coxiella burnetii (Cb) may utilize this approach to establish an optimized vacuole within a host cell. Previous methods employed to identify changes in host cell cycle induced by Cb infection have been unsuccessful in detecting a cell cycle modulation phenotype. Here we describe flow cytometric analysis to test impact on host cell cycle by sorting infected from uninfected cells and analyzing the infected population for phenotypes. This allowed us to detect subtle cell cycle manipulation induced by Cb, which were undetectable in unsorted culture. Two cell cycle modulation phenotypes were then characterized. The first detects the DNA content of each cell to determine the cell cycle phase, G0/G1, S, or G2/M. The distribution of the host cell cycle phases of each population was then computationally modeled and the change in phase distribution was compared for significant differences. Here we show that the Cb infected cells have a significantly different distribution compared to the uninfected, with a greater percentage of cells in S-phase compared to uninfected from Day 1 through Day 7 post-infection. The second screen tested the percentage of infected cells undergoing mitosis at late (>5 days post infection) time points by staining for phosphorylated-histone3 (pH3), a mitotic marker. We consistently found that both synchronized and unsynchronized infected cells undergo more frequent mitosis than uninfected cells at these late time points. These results indicate novel cell cycle modulation phenotypes induced by Cb infection and suggest cell cycle modulation may be required for CCV establishment and maturation. Additionally, initial data suggests that the S-phase phenotype is Dot/Icm-dependent and tests the potential for CBU388 to contribute to this cell cycle modulation.

P70

Functional characterization of Coxiella burnetii nuclear-targeted effectors during infection

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Coxiella burnetii is an obligate intracellular pathogen which causes the zoonotic disease Q Fever. *C. burnetii* develops in a specialized intracellular niche within the host endocytic pathway, where it is resistant to lysosomal degradation. Infected cells exhibit profound changes in cellular morphology, physiology, and transcriptional activity in response to *C. burnetii*. Host-targeted bacterial effectors delivered by the Type 4 Secretion System (T4SS) are hypothesized to be driving agents of these changes; however, functional characterization of putative effectors without existing transposon mutants has been hindered by difficult genetic tools to generate whole gene knockouts to use for such studies. More recent advances in the genetic tools available have allowed for targeted gene knockdown in *C. burnetii*. We engineered mutant *C. burnetii* using both antisense RNA and CRISPRi mediated knockdown of effectors localizing to the host nucleus when ectopically expressed. We have demonstrated that knockdown of CBU1314 results in an altered growth phenotype in infected cells, but not in axenic growth media. Here we also profile the host transcriptional response to CBU1314 knockdown and uncover a potential immunomodulatory role during *C. burnetii* infection. The successful application of two different knockdown systems has established a platform to target other *C. burnetii* nuclear effectors lacking transposon mutants to assign putative functions during infection. The functional characterization of nuclear- targeted effectors opens the door to mechanistic understanding of bacterial-host interaction in this important infection.

P71

Role of lipid droplets and prostaglandinE2 in Coxiella burnetii intracellular growth.

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Coxiella burnetii is an obligate intracellular bacterium that typically infects livestock. In humans, inhalation of *Coxiella* results in Q fever presented as atypical pneumonia which may relapse after a prolonged latent period leading to potentially fatal endocarditis. Our goal is to determine the mechanisms *Coxiella* employs to survive long-term in the host. While the bacterium initially infects alveolar macrophages, in endocarditis patients *Coxiella* is found in foamy macrophages that are rich in neutral lipid storage organelles called lipid droplets (LDs). Our previous studies show that *Coxiella* manipulates host LD metabolism via the Type 4 Secretion System (T4SS), a major virulence factor which secretes bacterial effector proteins into the host cell cytoplasm to manipulate cellular processes. Additionally, blocking LD breakdown almost completely inhibits bacterial growth suggesting that LD-derived lipids are critical for *Coxiella's* intracellular survival. LD breakdown releases arachidonic acids, precursors for the lipid immune mediator prostaglandin E2 (PGE2) which promotes an immunosuppressive environment within alveolar macrophages. We hypothesize that *Coxiella* manipulates host cell LD metabolism to promote a PGE2-mediated immunosuppressive environment and survive long-term in the host. To test this, we quantified PGE2 production during infection with and without LD breakdown inhibitor, Atglistatin. ELISA at 24- and 48-hours post-infection showed a LD breakdown-dependent significant increase in PGE2 levels. Fluorescence microscopy revealed that blocking PGE2 production using FDA-approved COX-2 inhibitors significantly decreased bacterial intracellular growth which was rescued with addition of exogenous PGE2. To directly assess the effect of PGE2, we treated infected macrophages with a PGE2-specific mPGES-1 inhibitor. Decreased *Coxiella* growth in mPGES-1-treated cells which was rescued with PGE2 suggests the importance of PGE2 during *Coxiella* infection. Our studies show that PGE2 promotes *Coxiella* survival in macrophages. Ongoing studies are identifying the correlation between LDs and PGE2 production and the contribution of LDs to immunosuppression during *Coxiella* infection.

P72

Growth characteristics of contemporary *Coxiella* strains *in vitro:* adventuring beyond Nine Mile

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Coxiella burnetii, the causative agent of Q fever in humans, is cultivated in vitro using methods which include tissue culture, axenic media, and embryonated eggs. Differences in growth between sequence types in vivo and in axenic media have been documented; however, studies characterizing these growth differences in cell culture are lacking. Current in vitro growth curve data has been based primarily on the Nine Mile Phase 1 strain (9Mi 1), which behaves differently in animal models compared to contemporary strains. Therefore, we evaluated the growth dynamics of the three predominant *C. burnetii* sequence types currently found in the United States in a variety of mammalian host cells. *C. burnetii* strains evaluated belong to sequence types ST8 (GP-CO1 and PB-CA2), ST16/26 (9Mi 1 and HPF-GA1), and ST20 (CM-SC1 and ES-FL1). These strains were grown in RK-13 (rabbit kidney epithelial cells) and THP-1 (human macrophage-like cells). Growth was monitored for 12 days revealing that 9Mi 1 grows extremely well in both cell types, while the contemporary strains demonstrated reduced growth that varied by cell type. Specifically, by day 12, 9Mi 1 had increased 83.69-fold in THP-1 cells and 113.82-fold in RK-13 cells, whereas the ST8 strain GP-CO1 only increased 0.16-fold in THP-1 and 6.58-fold in RK-13. By contrast, the ST20 isolates CM-SC1 and ES-FL1 grew better in THP-1 cells, increasing 6.55-fold and 3.27-fold, compared to 0.74-fold and 1.20-fold increases in RK-13 cells, respectively. Additionally, the contemporary ST16/26 isolate HPF-GA1 showed slower growth relative to 9Mi 1, with an increase of 14.82-fold in RK-13 and 8.69-fold in THP-1 cells. These data suggest that findings specific to the 9Mi 1 strain may not be broadly applicable to all *C. burnetii* isolates and that additional cell types should be explored for enhanced cultivation methods that target a broad range of *C. burnetii* isolates.

P73

Expression and Upregulation of de novo Folate Biosynthesis Pathway Genes of *Rickettsia monacensis* strain Humboldt in Engorged Larvae of *Ixodes pacificus*

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The nature of symbiotic relationships between ticks and their endosymbionts is unclear. However, evidence suggests a nutritional interaction between the endosymbionts and ticks. Our lab has identified a complete de novo folate biosynthesis pathway (including genes of folA, folC, folE, folKP, ptpS) in Rickettsia monacensis strain Humboldt, the endosymbiont of Ixodes pacificus. We hypothesized that folate genes are expressed and upregulated in engorged ticks to support the rapid growth and division of host cells required for the development and maturation of I. pacificus. Ticks were fed on New Zealand white rabbits. Total RNA and DNA were extracted from flat and engorged larvae, and gene expression of the folate genes of strain Humboldt was quantified by quantitative reverse transcription PCR. The genomic DNA of strain Humboldt was used to normalize the expression of genes. The average ratio of mRNA vs DNA for folA, folC, folE, folKP, and ptpS gene in engorged larvae was 7.32±4.27, 0.04±0.03, 0.11±0.06, 0.03±0.00, and 0.009±0.008, respectively. While the expressions of the folC, folKP, and ptpS of strain Humboldt were not detected in flat larvae, the average ratio of folA/DNA and folE/DNA of strain Humboldt in flat larvae is 0.53±0.48 and 0.04±0.04, respectively. Compared with flat larvae, the fold increase for folA and folE transcript of strain Humboldt in engorged larvae is 13.3 and 2.6. Mann Whitney U test showed a significant increase in the gene expression level of folA gene of strain Humboldt ($p<0.05$) in the engorged larvae. However, there is no significant increase in gene expression for the folE gene between engorged and flat larvae. Our data suggest the expression and upregulation of some folate genes after feeding may enable the tick to synthesize and metabolize folate more efficiently to support the tick's rapid growth.

P74

Tissue Localization and Profiling of Bacterial Symbionts in the Invasive Asian Longhorned Tick, *Haemaphysalis longicornis*

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The Asian Longhorned tick, *Haemaphysalis longicornis*, is native to eastern Asia but was first detected in the United States in 2017. Established populations of *H. longicornis* have since been detected in 18 states, and ecological niche models predict that the species will continue to expand its geographic range in North America. Intriguingly, all *H. longicornis* collected in North America are morphologically female and reproduce by parthenogenesis. Our group and others demonstrated that this invasive tick is capable of acquiring and transmitting tick-borne pathogens endemic to North America, including *Rickettsia rickettsii*, Powassan virus, and Heartland virus. In addition to pathogens, ticks harbor a broad range of commensal microorganisms. It has been shown that the bacterial community in ticks, even if transient, can impact tick fitness, development, reproduction, and vector competence. Thus, the objective of this project was tissue localization and determination of major symbionts in a laboratory colony of *H. longicornis* that reproduce by parthenogenesis. High-throughput sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene was performed to investigate bacterial abundance and diversity in adult female *H. longicornis*. The bacterial population structure was assessed in individual *H. longicornis* females statically incubated at different temperatures (7, 22, and 32°C). These ticks exhibited varying bacterial community composition and diversity across the different incubation temperatures. *Coxiella* was the dominant bacterial genus, while the *Rickettsia* genus was not represented in whole, individually processed *H. longicornis*. 16S rRNA sequencing of salivary glands, midgut, and ovaries from *H. longicornis* females is currently underway. Additionally, RNA *in situ* hybridization will be used to visualize the localization and spatial distribution of specific bacterial genera within tick organs. Together, these findings will lay the foundation for future studies examining the interactions between *H. longicornis*, commensal microbes, and pathogens.

LATE-BREAKING POSTERS

P75

The genetic landscape of *Ixodes scapularis* hemocytes

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The blacklegged tick *Ixodes scapularis* is a predominant arthropod vector in the United States and transmits several pathogens of public health and veterinary concern, including the Lyme disease spirochete *Borrelia burgdorferi* and the rickettsial agent *Anaplasma phagocytophilum*. Ticks are armed with specialized immune cells referred to as hemocytes, which are known for their capacity to elicit humoral and cellular responses. In other arthropods, these cells play roles in immunity, signaling, molting and development, clearing of apoptotic cells and transport of molecules. However, the full functional diversity, molecular characterization, and cellular differentiation of tick hemocytes remain elusive. Here, we employed a bulk and single cell RNA sequencing approach using hemocytes collected from unfed, engorged, *A. phagocytophilum*- and *B. burgdorferi*-infected ticks to characterize their response to feeding and microbial insult. We revealed that blood-feeding induces drastic changes in the abundance and gene expression profiles of hemocyte subpopulations. Specifically, we observed enrichment of immune and non-immune functions, including metabolism, cell growth/proliferation and molting/development, suggesting that hemocyte subtypes respond to metabolic alterations and may play a crucial role in tick physiology. We identified markers for each cluster (e.g., *hemocytin* and *astakine*), predicted hemocyte ontogeny lineages and uncovered differentially expressed genes during infection. Additionally, we manipulated the ratio of hemocyte subpopulations and the expression of marker genes *in vivo* and demonstrated alterations in bacterial acquisition, feeding, and molting capacity. Collectively, our work emphasizes the complexity of *I. scapularis* hemocytes, providing novel insight into arthropod immunity outside Pancrustacea.

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Assessment of Susceptibility to *Rickettsia conorii* Infection Using the Collaborative Cross Mouse Model

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Identification of the genetic and molecular mechanisms governing immunity against intracellular bacteria is imperative for understanding the host-pathogen-interplay and forms the basis for the development of therapeutic countermeasures. Previous attempts at increasing our understanding of this topic have relied on targeted interruption of individual genes or analysis genetic variability in natural populations. The Collaborative Cross (CC) mouse resource involves a cohort of recombinant-inbred lines generated by randomizing the genetic diversity of existing inbred mouse resources. Unlike outbred animal models, each CC line reproducibly exhibits distinct phenotypes of infection susceptibility and immune profiles. Using a murine model of *Rickettsia conorii* infection with well-established phenotypic differences in susceptibility to infection, we have screened 41 CC mouse lines to assess disease phenotype (bacterial load, weight loss, body temperature, survival) to identify baseline susceptibility and prepare for immunophenotypic and genetic analysis of susceptibility to infection. The various CC lines exhibited extraordinarily divergent susceptibility to lethal *Rickettsia* infection with some lines exhibiting little to no morbidity after challenge with 4 lethal doses of intravenous *Rickettsia conorii.* Computational data integration and bioinformatics tools (machine learning) will be applied to establish the immune landscape of Rickettsia-specific immune responses to identify genetic and immune correlates that govern disease phenotype of each CC line.

P77

Hepatocyte-Specific Regulation of Autophagy and Inflammasome Activation during Infection with Intracellular Bacteria Targeting Liver

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Hepatocytes, the major parenchymal cells in the liver, are highly important in protein synthesis, metabolism, and host response to infection. Ehrlichia is an obligate intracellular bacterial pathogen that target liver and cause potential fatal liver failure and sepsis. We have previously shown that fatal Ehrlichia infection induces liver damage via activation of deleterious MyD88-dependent caspase 11-mediated inflammasome pathways as well as inhibition of autophagy in macrophages. Although liver-resident macrophages are main target cells for Ehrlichia, Ehrlichia is also able to infect hepatocytes (HCs). However, the contribution of HCs to regulation of inflammasomes and autophagy remains elusive. We examined here the role of MyD88 signaling in the response of HCs to highly virulent Ixodes ovatus Ehrlichia (IOE), which causes fatal liver damage in mice, using primary cells from wild type and MyD88-/-mice and pharmacologic inhibitors of MyD88 in murine HC cell line. Similar to macrophages, MyD88 signaling in HCs resulted in deleterious caspase 11 activation, secretion of high group mobility box 1 (HMGB1), IL-6 production, and inflammatory cell death. Unlike macrophages, MyD88 signaling in IOE-infected HCs attenuated caspase-1 activation. Notably, MyD88 signaling promoted autophagy induction but blocked flux, which was associated with activation of mammalian target of rapamycin complex 1 (mTORC1, negative regulator of autophagy), and controlled bacterial replication. Mechanistically, blocking mTORC1 activation by rapamycin in IOE-infected HCs resulted in significant induction of autophagy only in the absence, but not in the presence, of MYD88 signaling, suggesting that MyD88 abrogate mTORC1-mediated inhibition of autophagy in HCs. Together, our findings demonstrate, for the first time, that regulation of autophagy and caspase 1 mediated canonical inflammasome pathway by MYD88 during fatal Ehrlichia infection is paradoxical to what occurs in macrophages. Understanding HCs-specific signaling is critical for development of new therapeutic approaches against virulent infectious agents targeting liver such as Ehrlichia.

P78

Development of a rapid antigen detection test as a potential diagnostic assay for fatal spotted fever rickettsioses

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Tick-borne spotted fever rickettsioses (SFR) can be life-threatening, with most mortality resulting from lack of a timely and specific diagnostic assay. We previously reported the identification and detection of a diagnostic biomarker, the putative N-acetylmuramoyl-l-alanine amidase RC0497, by mass spectrometry (MS) both in experimentally infected animals and in patients. Here, we sought to develop a lateral-flow-assay (LFA) for rapid detection of RC0497 as a potentially early diagnostic assay for fatal SFR. By stable isotope dilution (SID)-parallel reaction monitoring (PRM)-MS, we found that the concentrations of RC0497 in sera of *Rickettsia rickettsii*-infected guinea pigs and *R. conorii*-infected mice ranged from 0.1 to 1.1 ng/ml. Using europium chelate nanoparticle reporters, we developed a prototype LFA and evaluated the test by a panel of serum samples of experimentally infected animals. Interestingly, 19 of 20 (95%) serum samples from *R. rickettsii*-infected guinea pigs and *R. conorii*-infected mice, showed positive results of test line (TL)/control line (CL). Only one infected sample showed positive result of TL, but not CL. All the serum specimens from mock-infected animals showed negative results, suggesting that the specificity of the test was 100%. Mice infected with a lethal dose of *R. conorii* treated with doxycycline on day 3 post infection (p.i.), when RC0497 was detected by LFA, showed significantly diminished rickettsial loads in tissues on day 5 p.i.. comparable to those in sublethal infection group. These results suggest that detection of RC0497 by a europium LFA is timely for guiding the treatment to improve the infection outcome. This work, for the first time, shows promise for a rapid and easy-to-use platform offering a timely point-of-care diagnostic assay for severe SFR.

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Neisseria gonorrhoeae drives *Chlamydia trachomatis* into a persistent state during *in vitro* co-infection

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Chlamydia trachomatis and *Neisseria gonorrhoeae* are the top two most prevalent bacterial sexually transmitted diseases globally. In addition to mono-infections, co-infections are frequently reported in human patients. We hypothesized that co-infection of these two pathogens would increase bacterial load when grown *in vitro*. *C. trachomatis* is an obligate intracellular parasite with a biphasic developmental cycle. The infectious elementary body (EB) converts to a reticulate body (RB) and replicates within an inclusion in the host cell. RBs differentiate back to EBs and exit the host cell by lysis or extrusion. We infected epithelial cells with *C. trachomatis* for 12h, then challenged with *N. gonorrhoeae* for an additional 12h. To our surprise we found that gonococcal growth was unaffected by the presence of *C. trachomatis*, but *C. trachomatis* entered a stress stimulated response described as “persistence”, a reversible dormant state enabling survival in sub-optimal growth conditions. We observed persistence whether *N. gonorrhoeae* was grown directly on the eukaryotic cell surface, or in a transwell above *C. trachomatis*-infected cells. The phenotype was not alleviated by nutritional supplementation, but required live, actively growing *N. gonorrhoeae*. We conducted an RNA-seq analysis of the *C. trachomatis* transcriptome during co-infection and found that genes up or down regulated in our study were modulated similarly to genes in previously published persistence studies. Of note, chlamydial heat shock protein 60 (cHsp60), was upregulated almost 4-fold. Antiserum to this protein is increased in women with pelvic inflammatory disease meaning that co-infections may be more damaging than appreciated. Overall, we showed that *C. trachomatis* enters a dormant state during *in vitro* co-infection with *N. gonorrhoeae* and this is induced by the modulation of the shared medium by the gonococci. The effect on the chlamydial transcriptome is extensive and increases transcription of a protein associated with poor health outcomes for women.

P80

Evasion of Host Antioxidative Response via Disruption of NRF2 Signaling in Fatal *Ehrlichia* -Induced Liver Injury

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Background and Aims: *Ehrlichia* are Gram negative obligate intracellular bacterium that cause human monocytotropic ehrlichiosis (HME). HME is characterized by acute liver damage and inflammation that may progress to fatal toxic shock. We previously showed that fatal ehrlichiosis is due to deleterious activation of inflammasome pathways, which causes excessive inflammation and liver injury. Mammalian cells have developed mechanisms to control oxidative stress via regulation of nuclear factor erythroid 2 related 2 (NRF2) signaling. However, the contribution of NRF2 signaling to *Ehrlichia*-induced inflammasome activation and liver damage remains elusive. In this study, we investigated the role of NRF2 signaling in ehrlichiosis.

Methods: We infected murine cell line AML12 (HCs) and C57BL/6 mice with virulent Ixodes ovatus *Ehrlichia* (IOE, AKA E. japonica), and examined the NRF2 signaling.

Results: Our results revealed that virulent IOE inhibited NRF2 signaling in liver tissue of infected mice and in HCs as evidenced by downregulation of NRF2 expression, and downstream target GPX4, as well as decreased NRF2 nuclear translocation, a key step in NRF2 activation. This was associated with activation of inflammasome pathway, accumulation of reactive oxygen species (ROS), mitochondrial dysfunction, and endoplasmic reticulum (ER) stress. Mechanistically, treatment of IOE-infected HCs with the antioxidant 3H-1,2-Dithiole-3-Thione (D3T), that induce NRF2 activation attenuated oxidative stress and inflammasome activation, and restored cell viability. Notably, treatment of IOE-infected mice with D3T resulted in attenuated liver pathology and inflammation, enhanced bacterial clearance, prolonged survival, and resistance to fatal ehrlichiosis.

Conclusions: Our study reveals, for the first time, that targeting anti-oxidative signaling pathway is a key approach in the treatment of severe and potential *Ehrlichia*-induced acute liver injury.

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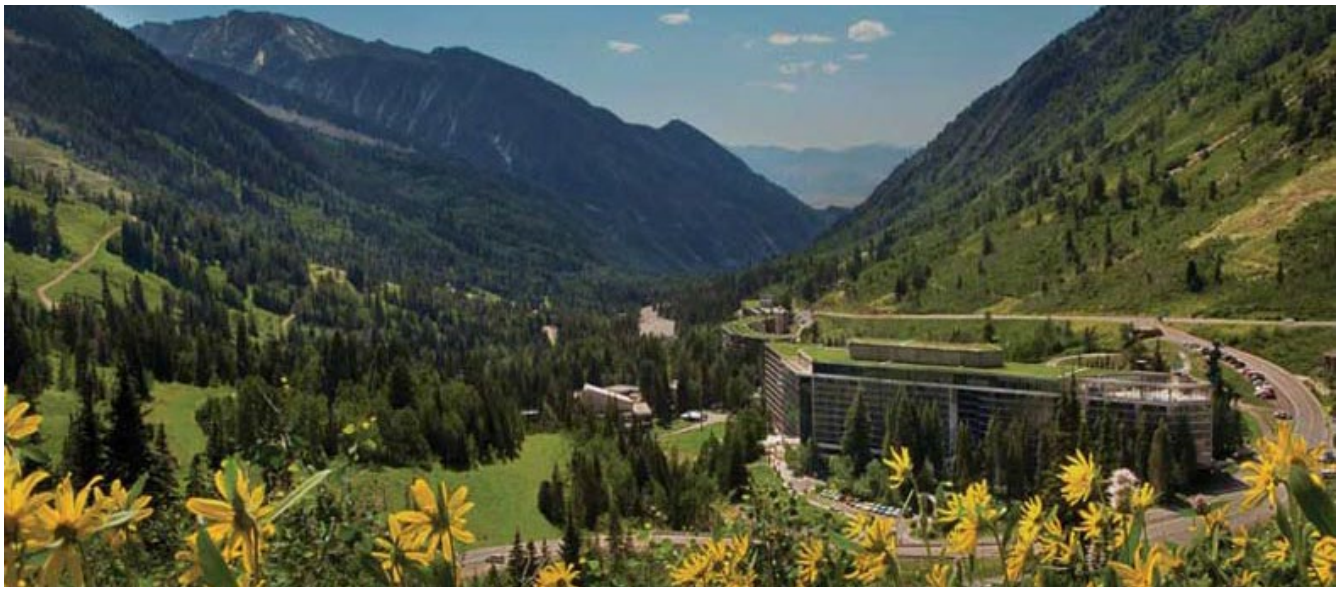

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