29th Meeting of the American Society for Rickettsiology

June 16-19, 2018
Hyatt Regency Milwaukee
Milwaukee, WI

Sponsored by
Funding for this conference was made possible [in part] by R13 AI126727-01 from the 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. Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.
# 2018 Schedule-at-a-Glance

## Saturday, June 16th

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tr>
<td>3-6:45 p.m.</td>
<td>Conference Registration – <em>Regency Ballroom Pre-function</em></td>
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<tr>
<td></td>
<td>Oral presentation drop-off</td>
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<tr>
<td>6-8 p.m.</td>
<td>Welcome Reception – <em>Regency Ballroom</em></td>
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<tr>
<td>7-8 p.m.</td>
<td>Invited Historical Lecture – <em>Regency Ballroom</em></td>
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## Sunday, June 17th

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<th>Time</th>
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<td>7-8 a.m.</td>
<td>Breakfast Buffet – <em>Vue Room 21st Floor</em></td>
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<td></td>
<td>Poster Session A Setup – <em>Regency Ballroom AD</em></td>
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<tr>
<td></td>
<td>Conference Registration – <em>Regency Pre-function</em></td>
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<tr>
<td></td>
<td>Oral presentation drop-off</td>
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<tr>
<td>8 a.m.</td>
<td>Opening and Welcome Remarks – <em>Regency Ballroom B</em></td>
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<tr>
<td>8-9:40 a.m.</td>
<td>Workshop Session 1: Genetic Transformation and Cellular Microbiology in Rickettsia – <em>Regency Ballroom B</em></td>
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<tr>
<td>9:40-10:15 a.m.</td>
<td>Poster Session A and Break – <em>Regency Ballroom AD</em></td>
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<tr>
<td>10:15-12 p.m.</td>
<td>Workshop Session 2: Axenic Culture &amp; Genetic Transformation in Coxiella – <em>Regency B</em></td>
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<tr>
<td>12-2:15 p.m.</td>
<td>Buffet Luncheon – <em>Vue Room 21st Floor</em></td>
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<tr>
<td></td>
<td>Lunch and Learn Presentation (optional) 12:30-1:15 p.m.</td>
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<tr>
<td></td>
<td>Poster Session A – <em>Regency Ballroom AD</em></td>
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<tr>
<td>3-4 p.m.</td>
<td>Concurrent Scientific Sessions:</td>
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<tr>
<td></td>
<td>4: Genomics, Transcriptomics, and Proteomics – <em>Regency Ballroom B</em></td>
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<td></td>
<td>5: Epidemiology and Ecology – <em>Regency Ballroom C</em></td>
</tr>
<tr>
<td>4-5 p.m.</td>
<td>ASR Business Meeting and Election of New Officers – <em>Regency Ballroom B</em></td>
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<tr>
<td>5-6:30 p.m.</td>
<td>Poster Session A and Pre-Dinner Reception – <em>Regency Ballroom AD</em></td>
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<tr>
<td>6:30-8 p.m.</td>
<td>Gala Dinner – <em>Vue Room 21st Floor</em></td>
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## Monday, June 18th

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<tr>
<td>7-8 a.m.</td>
<td>Breakfast Buffet – 2nd <em>Floor Atrium</em></td>
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<td></td>
<td>Poster Session B Setup – <em>Regency Ballroom AD</em></td>
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<tr>
<td>8-10 a.m.</td>
<td>Workshop Session 6: Outside of the Box; CRISPR, Host-associated Bacteria, Chlamydia – <em>Regency Ballroom B</em></td>
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<tr>
<td>10-10:45 a.m.</td>
<td>Poster Session B and Break – <em>Regency Ballroom AD</em></td>
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<tr>
<td>10:45-12:00 p.m.</td>
<td>Concurrent Scientific Sessions:</td>
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<tr>
<td></td>
<td>7: Immunity and Vaccine Development – <em>Regency Ballroom B</em></td>
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<td></td>
<td>8: Pathogenesis, Pathophysiology, and Cell Biology I – <em>Regency Ballroom C</em></td>
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<tr>
<td>12-6:00 p.m.</td>
<td>Free afternoon to explore!</td>
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<tr>
<td>6-8 p.m.</td>
<td>Poster Session B and Cocktail Reception – <em>Regency Ballroom AD</em></td>
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## Tuesday, June 19th

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<tr>
<td>7-8 a.m.</td>
<td>Breakfast Buffet – 2nd <em>Floor Atrium</em></td>
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<tr>
<td>8-10 a.m.</td>
<td>Concurrent Scientific Sessions:</td>
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<tr>
<td></td>
<td>9: Host, Vector, and Pathogen Interactions I – <em>Regency Ballroom B</em></td>
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<tr>
<td></td>
<td>10: Pathogenesis/Pathophysiology/Cell Biology II – <em>Regency Ballroom C</em></td>
</tr>
<tr>
<td>10-10:30 a.m.</td>
<td>Refreshment Break – <em>Regency Ballroom AD</em></td>
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<tr>
<td>10:30-12:00 p.m.</td>
<td>Concurrent Scientific Sessions:</td>
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<tr>
<td></td>
<td>11: Host, Vector, Pathogen Interactions II – <em>Regency Ballroom B</em></td>
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<td></td>
<td>12: Infection, Diagnosis &amp; Treatment – <em>Regency Ballroom C</em></td>
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<td>12-12:05 p.m.</td>
<td>Closing Remarks and Adjournment – <em>Regency Ballroom B</em></td>
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Meeting Room Map

Vue Room (21st Floor)  Second Floor

- Oral Presentations ~ Regency B and C
- Poster Presentations ~ Regency AD
- Meals ~ Vue Room and 2nd Floor Atrium
29th Meeting of the American Society for Rickettsiology

June 16-19, 2018
Hyatt Regency Milwaukee, Milwaukee Wisconsin

Welcome Reception will be held on Saturday, June 16th
6:00 – 8:00 p.m. in the Regency Ballroom.

Gala Dinner will be held on Sunday, June 17th
6:30 – 8:00 p.m. in the Vue Room, following a reception and poster viewing.

Historical Lecturer: Dr. Arthur Allen

His newest book is entitled The Fantastic Laboratory of Dr. Weigl: How Two Brave Scientists Battled Typhus and Sabotaged the Nazis.
Saturday, June 16th

3:00 – 6:45 p.m.
Conference Registration & Check-In – Regency Prefunction

6:00 – 8:00 p.m.
Welcome Reception – Regency Ballroom CD
Hors d’oeuvres and two drink tickets per guest provided to be used at the full cash bar

7:00 – 8:00 p.m.
Invited Historical Lecture – Regency Ballroom CD
Dr. Arthur Allen – “Rudolf Weigl and Ludwik Fleck: Vaccinating against Typhus, Vaccines against Nazism”

Sunday, June 17th

7:00 – 8:00 a.m.
Hot Breakfast Buffet – Vue Room 21st Floor
Conference Registration – Regency Prefunction

Oral presentation check-in and drop-off – Registration Desk, Regency Prefunction
Poster Session A Setup – Regency AD

8:00 a.m.
Opening and Welcome Remarks – Regency B
Dr. J. Stephen Dumler, Uniformed Services University of the Health Sciences, Walter Reed National Military Medica Center

8:00 – 9:40 a.m.
Workshop Session 1: Genetic Transformation and Cellular Microbiology in Rickettsia
Session Chair: Uli Munderloh

Regency Ballroom B

8:05 a.m.  Abstract #1: Beating the Odds: Challenges and Perspectives in Random Mutagenesis of Rickettsiales
Plenary Overview: Ulrike Munderloh

8:50 a.m.  Abstract #2: Challenges (and Some Successes) in Spotted Fever Group Rickettsia Genetic Manipulation
Key Abstract: Sean Riley
9:10 a.m.  Abstract #3: Forward Genetic Analysis Identifies Factors Crucial for Rickettsia parkeri Cell-to-Cell Spread
Invited Short Talk: Rebecca Lamason

9:25 a.m.  Abstract #4: Dokkaebi Transposon Mutagenesis Identifies Genes Important for R. conorii Life-Cycle
Hwan Keun Kim

9:40 – 10:15 a.m.
Poster Session A: Epidemiology, Ecology, Infection, Diagnosis & Treatment
Refreshments Provided

Regency Ballroom AD

Abstract #5: Genetic Diversity of Bartonella spp. in Wild Mammals & Ectoparasites in Brazilian Pantanal
Marcos Andre

Abstract #6: Evidence and Molecular Characterization of Bartonella spp. in Bats in Brazil
Marcos Andre

Abstract #7: Q Fever Diagnostic at Poti and Gori Laboratory Support Stations
Eka Korchashvili

Abstract #8: Complete Blood Count Abnormalities in Bartonella Species Seroreactive Dogs in the United States
Anton Mestek

Abstract #9: Molecular Detection of Bartonella in South American Fur Seals (Arctocephalus australis) from Chilean Patagonia
Pedro Bittencourt

Abstract #10: Molecular Detection and Characterization of Bartonella spp. and Rickettsia spp. in Ectoparasites from Bats in Brazil
Marcos Andre

Abstract #11: Molecular Detection of Rickettsia spp. and Coxiella burnetii in Ruminants and Dogs from Selected Areas of Luzon Island, Philippines
Remil Galay

Abstract #12: Filling in the Gaps: A Survey of Ticks (Acari: Ixodidae) across the Northern Counties of Missouri
William Nicholson

Abstract #13: Prevalence and Strain Diversity of Anaplasma marginale in Kansas Cattle Herds
Tippawan Anantatat

Abstract #14: Molecular Detection of Anaplasma phagocytophilum in a Patient and a Tick Collected from the Patient in South Korea
Sungdo Park

Abstract #15: Molecular Identification of Anaplasma phagocytophilum Infection in Holstein Cattle in the Republic of Korea
Kyoung-Seong Choi
Abstract #16: Serologic Survey of *Ehrlichia canis* Infection in Humans and Dogs in the State of Cauca (Colombia, South America)  
Jere McBride

Abstract #17: Big Data - Worldwide SNAP 4Dx® and SNAP 4Dx Plus® Clinic-based Serologic Survey of Tick-borne Diseases in Dogs  
Jesse Buch

Abstract #18: Seasonal and Geographical Distribution of Scrub Typhus in South Western India  
Indira Bairy

Abstract #19: The Role of Cofeeding Arthropods in Transmission of an Emerging Rickettsial Pathogen  
Chanida Fongsaran

Abstract #20: Epidemiologic Profile and Clinical Course of *Rickettsiosis* Cases in Southern Mexico, 2015-2017  
Karla Dzul-Rosado

Abstract #21: Suburban Focus of *Rickettsiosis* in Yucatan, Mexico  
Karla Dzul-Rosado

Abstract #22: First Report of Detection of *Rickettsia* Pathogenic Bacteria in Trombiculid Chiggers from Thailand  
Sirima Wongwairot

Joy Hecht

Abstract #24: *Rickettsia* Asembonensis Causing Febrile Disease in Southern Thailand, 2016-2018  
Wuttikon Rodkvamtook

Abstract #25: Differentiation of *Rickettsia typhi*, *R. felis*, and *R. felis*-like Organisms via Restriction Fragment Length Polymorphism Analysis  
Bethany Quade

Abstract #26: Introduction of an Informational Website for the Rickettsial Disease Scrub Typhus  
Paul Fuerst

Abstract #27: Rickettsial Infections among Cats and Cat Fleas in Riverside County, California  
Kristin Mullins

Abstract #28: Febrile Exantheme Syndrisme Originated by *Rickettsia* in Tropical Regions: Clinical and Sociocultural Differences from Viral Diseases  
Cesar Lugo

Abstract #29: Rickettsiae within the Fleas of Feral Cats in Galveston, Texas  
Lucas Blanton

Abstract #30: Active Surveillance for Vectors and Reservoirs of *Rickettsiosis* and Scrub Typhus in Kut Island, Gulf of Thailand  
Jariyanart Gaywee

Abstract #31: Identifying and Diagnosing *Bartonella* Infections in Morocco  
Zineb Chekli
Abstract #32: Survival of *Coxiella burnetii* in a Laboratory Generated Aerosol
Angela Essex-Lopresti

Abstract #33: The Effect of pH on Antibiotic Efficacy against *Coxiella burnetii* in Axenic Media
Cody Smith

Abstract #34: *Bartonella vinsonii* subsp. *berkhoffii* and *B. henselae* in Dogs from Chile
Ananda Müller

Abstract #35: First Case Report of Human Granulocytic Anaplasmosis in Mexico with Serological and Molecular Evidence
Carolina Sosa-Gutierrez

Abstract #36: The Occurrence of Human Granulocytic Anaplasmosis in South Korea during 2015-2017
Hae Kyung Lee

Abstract #37: Comprehensive Screening Of Vector-Borne Infections: Accuracy Of Rapid Tests
Chandra Ramaswamy

Abstract #38: Proteome-wide Identification of Novel *Ehrlichia* HECT-like E3 Ligases
Bing Zhu

Abstract #39: The Case for Valid Rickettsia IgM Assays
Lee Fuller

Abstract #40: Reliability of the Polymerase Chain Reaction for Detecting *Rickettsia* spp. and *Rickettsia rickettsii* Infection in Patients from Sonora, Mexico
Marcia Leyva Gastelum

Abstract #41: Identification and Characterization of a Novel Adhering Receptor for Spotted Fever Group *Rickettsiae*
Bin Gong

Abstract #42: Development of a *Rickettsia* 364D-specific TaqMan Assay
Sandor Karpathy

Abstract #43: Employing FDA-approved Compounds to Develop Host-targeted Anti-*Rickettsia* Therapy
Sean Riley

Abstract #44: Serological Assessment of *Rickettsia rickettsii*, *R. typhi*, and *R. parkeri* Species Reactivity in Patients with Suspected Rocky Mountain Spotted Fever
Cecilia Kato

Abstract #45: National Capacity for Rickettsia Molecular Detection
Cecilia Kato

Abstract #46: Development of an *Orientia* Genus-specific Quantitative Real-time PCR Assay and the Detection of *Orientia* Species in DNA Preparations from *O. tsutsugamushi*, *Candidatus Orientia chuto*, and *Orientia* Species from Chile
Ju Jiang

Abstract #47: Modeling Spatial Distributions of Ticks and Tick-borne Diseases
Ram Raghavan
10:15 a.m. – 12:00 p.m.
Workshop Session 2: Axenic Culture and Genetic Transformation in *Coxiella*
Session Chair: James Samuel

**Regency Ballroom B**

10:15 a.m.  
Abstract #48: *Coxiella* Genetics: How Far Have We Come?  
Plenary Overview: Paul Beare

10:55 a.m.  
Abstract #49: A Nutritionally-defined Media Enables Analysis of *Coxiella burnetii* Physiology  
Invited Short Talk: Kelsi Sandoz

11:10 a.m.  
Abstract #50: Growth and Analysis of *Coxiella burnetii* Using Axenic Media  
Key Speaker: Gil Kersh

11:25 a.m.  
Abstract #51: Applying Evolutionary Insights to Control *Coxiella burnetii* Growth  
Invited Short Talk: Rahul Raghavan

11:40 a.m.  
Abstract #52: Development and Effective Utilization of Axenic Culture Tools in the Study of Bacterial Obligate Intracellular Parasites  
Key Speaker: Anders Omsland

12:00 – 2:15 p.m.
**Buffet Luncheon** – *Vue Room 21st Floor*

12:30 – 1:15 p.m.
**Lunch and Learn (optional)** – *Vue Room 21st Floor*
Dr. Liangbao Zheng, Scientific Review Officer, Infectious Diseases and Microbiology IRG, Centers for Scientific Review, NIH

12:30 – 2:15 p.m.
Poster Session A: Epidemiology, Ecology, Infection, Diagnosis & Treatment – *Regency Ballroom AD*  
*See above for poster details.*

2:15 – 2:55 p.m.
Workshop Session 3: Anaplasma and *Ehrlichia* - Vector Transmitted, Vacuolar Bacterial Genetic Manipulations  
Session Chair: Kelly Brayton

**Regency Ballroom B**

2:15 p.m.  
Abstract #53: Targeted Mutagenesis Broadly Applicable in *Ehrlichia* Species in Creating Mutations to Disrupt and Restore a Gene Activity, and also to Introduce Expression Tags in Proteins  
Plenary Overview: Roman Ganta
### 3:00 – 4:00 p.m.
Concurrent Scientific Sessions

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<th>4: Genomics, Transcriptomics, Proteomics</th>
<th>5: Epidemiology and Ecology</th>
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<td>Session Chair: Kelly Brayton</td>
<td>Session Chair: Lucas Blanton</td>
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### Regency Ballroom B

- **3:00 p.m.**
  - Abstract #54: Impact of Three Different Mutations in *Ehrlichia chaffeensis* in Altering the Global Gene Expression Patterns
  - Chandramouli Kondethimmanahalli

- **3:15 p.m.**
  - Abstract #55: The *Anaplasma marginale* msp2 Pseudogene Repertoire: Multistrain Genomic Analysis Provides Insights to Evolution and Fitness
  - Kelly Brayton

- **3:30 p.m.**
  - Abstract #56: Global Transcriptomics Analysis of Macrophages Infected by a Pathogen and a Non-pathogen SFG *Rickettsia* Reveals Differential Reprogramming of Host Signaling Pathways
  - Pedro Curto

- **4:45 p.m.**
  - Abstract #57: Direct Sequencing of *Rickettsia* Plasmidomes from Ticks
  - Arunachalam Ramaiah

### Regency Ballroom C

- **3:00 p.m.**
  - Abstract #58: Scrub Typhus Infection Burden in Gorakhpur District, Uttar Pradesh, India: A Community-based study during Lean & Peak Periods for Acute Encephalitis
  - Suchit Kamble

- **3:15 p.m.**
  - Abstract #59: Community-based Prevention of Rocky Mountain Spotted Fever, Sonora, Mexico
  - Gerardo Alvarez

- **3:30 p.m.**
  - Abstract #60: Glucose-6-phosphate Dehydrogenase Deficiency and Fatal Rocky Mountain Spotted Fever in Brazil and the United States
  - Chris Paddock

- **4:45 p.m.**
  - Abstract #61: Evaluation of Multiple Chlortetracycline-Medicated Mineral Formulations to Control Active Bovine Anaplasmosis in Endemic Cattle Herds
  - Kathryn Reif

### 4:00 – 5:00 p.m.
ASR Business Meeting and Election of New Officers – Regency B

### 5:00 – 6:30 p.m.
Pre-Dinner Reception and Poster Session A – Regency AD

*See above for poster details.*

### 6:30 – 8:00 p.m.
Gala Dinner – Vue Room

*Guests of attendees may join the Gala Dinner with purchase of a ticket from the registration table, $110.*
Monday, June 18th

7:00 – 8:00 a.m.
Hot Breakfast Buffet – 2nd Floor Atrium

Oral presentation check-in and drop-off – Registration Desk, Regency Prefunction
Poster Session B Setup – Regency AD

8:00 – 10:00 a.m.
Workshop Session 6: Outside of the Box - CRISPR, Host-associated Bacteria, Chlamydia
Session Chair: Paul Beare

Regency Ballroom B

8:00 a.m. Abstract #62: Genetic Manipulation Strategies for Chlamydia: Status, Strengths, and Limitations
Keynote Address: Scott Hefty

8:40 a.m. Abstract #63: Expanding the Bacterial Genetics Toolkit with CRISPR Interference
Plenary Overview: Jeremy Rock

9:20 a.m. Abstract #64: Advancing CRISPR-based Tools in Non-model Bacteria
Plenary Overview: Chase L. Beisel

10:00 – 10:45 a.m.
Poster Session B: Genomics, Transcriptomics, Proteomics, Host, Vector, Pathogen Interactions, Pathogenesis/Pathophysiology/Cell Biology, Immunity/Vaccine Development – Refreshments Provided

Regency Ballroom AD

Abstract #65: Whole Genome of "Rickettsia parkeri" strain Atlantic Rainforest-Like Isolated from a Colombian Tick
Andres Londono

Abstract #66: Time-dependent, Post-infection Changes of Serum Metabolites from Mice Challenged with Orientia tsutsugamushi-infected Mites
Chien-Chung Chao

Abstract #67: RARP2 of Rickettsia rickettsii is Transcribed Early and at High Levels
Tina Clark

Abstract #68: Rickettsial Shuttle Vectors With Expanded Host Range Reveal Mechanisms for Maintenance of the R. monacensis Plasmid pRM
Nicole Burkhardt

Abstract #69: Survey of Sheep Herds' Anaplasmataceae Bacteria in Senegal
Djiba Mamadou Lamine
Abstract #70: The Effect of Iron Starvation of Tick Cells on *A. marginale* Replication
Muna Solyman

Abstract #71: Neutralization of Ehrlichial transmission from Tick to Mammalian Cells by Anti-Ehrlichial Invasin Protein, EtpE Antibody
Kehraj Budachetri

Abstract #72: *Orientia tsutsugamushi* Negatively Regulates Major Histocompatibility Complex I Expression via Reduction of the Transcription Factor NLRC5
Kyle Rodino

Abstract #73: Assessment of the Pathogenicity of Symbiotic *Rickettsia* in Guinea Pigs
Alyssa Snellgrove

Abstract #74: Dynamics of Rickettsial Load in Tick Salivary Glands and Saliva during Tick Feeding
Chanakan Suwanbongkot

Abstract #75: Nuclear Trafficking of *Orientia tsutsugamushi* Effector Ank13
Haley Adcox

Abstract #76: Culture of *Rickettsia felis* Isolated from a Tick
Monika Danchenko

Abstract #77: Eschar Development in Mice during *Rickettsia parkeri* Infection by Tick Transmission
Tais B. Saito

Abstract #78: Screening of Ticks - Potential Vectors for Rickettsia in Azerbaijan
Yegana Sultanova

Abstract #79: A Preliminary Comparison of Five Assays for Detecting Past Exposure to *Coxiella burnetii* for Use Prior to Human Q Fever Vaccination
Stephen Graves

Abstract #80: Formalin-Inactivated *Coxiella burnetii* Phase I Vaccine Elicits Recruitment of Eosinophils to the Spleen
Lindsey Ledbetter

Abstract #81: Both MHC Class I and Class II Molecules are Required while MHC-I Appears to Play a Critical Role in Host Defense against Primary *Coxiella burnetii* Infection
Lindsey Ledbetter

Abstract #82: Efficacy of Intranasal Immunization with Whole-Cell Fixed *Coxiella burnetii* Nine Mile Phase I on Subsequent Pathogenesis Using a Guinea Pig Model
Edward Shaw

Abstract #83: Mechanism of a Mimetic Peptide Vaccine-induced Protective Immunity against Q Fever
Guoquan Zhang

Abstract #84: Designing and Evaluating Q Fever Vaccines in Mice and Guinea Pig Models of Aerosol Infection
Anthon Gregory

Abstract #85: Neutrophils and M1 Macrophages Contribute to Vascular Injury and Lung Pathogenesis during *Orientia tsutsugamushi* Infection
Brandon Trent
Abstract #86: Developing a Guinea Pig Model to Interrogate the Immune Response to Spotted Fever Rickettsiae
John Stokes

Abstract #87: Restriction Fragment Length Polymorphism Analysis of Orientia tsutsugamushi scaE and scaC
Munegowda Koralur

Abstract #88: A Type I Interferon/IL-10 axis induced by Orientia tsutsugamushi Infection Suppresses Antigen-specific T-cells and their Memory Responses
Hong-Il Kim

Abstract #89: Activation of ASC Inflammasome Driven by TLR4 Contributes to Host Immunity against R. australis Infection
Rong Fang

Abstract #90: A Novel elncRNA as Potential Regulator of CD8+ T-cells and Anti-rickettsial Immunity
Imran Chowdhury

Abstract #91: Exchange Protein Directly Activated by cAMP Plays a Critical Role in Fatal Rickettsioses
Bin Gong

Abstract #92: Investigation of Coxiella burnetii Phospholipid Metabolism
Chris Stead

Abstract #93: Inhibition of mTORC1 by Coxiella burnetii Promotes Replication within a Phagolysosome-like Vacuole
Charles Larson

Abstract #94: Actin Polymerization in the Endosomal Pathway, but not on the Coxiella-containing Vacuole, is Essential for Pathogen Growth
Heather Miller

Abstract #95: Coxiella burnetii Blocks IL-17 Signaling in Macrophages
Tatiana Clemente

Abstract #96: In Vitro and In Vivo Localization of the Hypothetical Membrane Protein CBU_1651
Brandon Luedtke

Abstract #97: A Tale of New Isolates: Responses of Mice to Aerosol Infection with Novel Isolates of C. burnetii
Rachael Priestley

Abstract #98: The Coxiella burnetii Type IV Effector CBU0794 Modulates Host Nuclear Processes
Sara Talmage

Abstract #99: Ehrlichia chaffeensis TRP120 HECT Ub Ligase Targets FBW7 for Degradation to Maintain NOTCH Signaling to Promote Infection
Jennifer Wang

Abstract #100: Characterization of the Eukaryotic Wnt Ligand Mimic Properties of Ehrlichia chaffeensis Effector TRP120
Madison Rogan

Abstract #101: Interaction of Apoptosis and Actin Cytoskeleton-Associated Host Proteins with Ehrlichia chaffeensis TRP75 Promotes Infection
Tian Luo
Abstract #102: A Genetic System for Creating Targeted Mutations to Disrupt and Restore Genes in *Ehrlichia chaffeensis* that is Broadly Applicable to Other Obligate Bacteria  
Ying Wang

Abstract #103: Sequence Determinants Spanning -10 Motif and Spacer Region Impacting *Ehrlichia chaffeensis* Sigma 32-Dependent Dnak Gene Promoter Activity  
Huitao Liu

Abstract #104: Differential Susceptibility of Laboratory Mice to *A. phagocytophilum* Infection  
Waheeda A. Naimi

Abstract #105: The Cell Wall of *Orientia tsutsugamushi* and Other *Rickettsiales* Species  
Sharanjeet Atwal

Abstract #106: Inactivation of Host EPAC1 Can Prevent Spotted Fever Group *Rickettsia* from Adhering to Vascular Luminal Surface  
Xi He

Abstract #107: Molecular, Biochemical and Cellular Characterization of Clinic Isolates of *Rickettsia sp.*  
Cesar Lugo

Abstract #108: MicroRNA-regulated Rickettsial Invasion into Host Endothelium via Fibroblast Growth Factor (FGF)-2 and its Receptor FGFR-1  
Abha Sahni

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**10:45 a.m. – 12:00 p.m.**  
Concurrent Scientific Sessions  

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<th>Regency Ballroom B</th>
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| **7: Immunity and Vaccine Development**  
Session Chair: Lindsey Ledbetter | **8: Pathogenesis, Pathophysiology, and Cell Biology I**  
Session Chair: Patrik Engstrom |

10:45 a.m.  
Abstract #109: Anaplasma-phagocytophilum-related Defects in CD8, NKT, and NK Lymphocyte Cytotoxicity  
Diana Scorpio

11:00 a.m.  
Abstract #110: Human Anti-OMP-1 Monoclonal Antibody Inhibits *Ehrlichia chaffeensis* Interactions  
Thangam Sudha Velayutham

11:15 a.m.  
Abstract #111: Immunization with a Recombinant Antigen Composed of Conserved Blocks from TSA56 Provides Broad Genotype Protection against Scrub Typhus  
Nam-Hyuk Cho

Abstract #114: Understanding the Significance of the Interactions between Spotted Fever Group *Rickettsia* and Mammalian Phagocytic Cells  
Juan Martinez

Abstract #115: Identification of Type IV Secretion Effectors that are Specifically Required for Replication of *Coxiella burnetii* in Primary Macrophages  
Elizabeth Case

Abstract #116: An *Ehrlichia* Type IV Secretion System Effector Etf-2 Binds to Active RAB5, and Delays Endosome Maturation  
Yasuko Rikihisa
11:30 a.m.  Abstract #112: Evaluation of T-cell Population Cytokine Signatures Associated with a Sub-lethal Mouse Model of Orientia tsutsugamushi and Comparison to Lethal and Non-lethal Mouse Models of O. tsutsugamushi  
Alison Fedrow

Abstract #117: Acid Sphingomyelinase is Essential for the Infection Cycles of Vacuole Adapted Pathogens  
Chelsea Cockburn

11:45 a.m.  Abstract #113: Rickettsia rickettsii Whole Cell Antigen Vaccine Offers Protection against Virulent Pathogen Infection Challenge  
Andy Alhassan

Abstract #118: Nuclear Import of Anaplasma Phagocytophilum AnkA Uses a Eukaryotic Code in N-terminal Ankyrin Repeats Known to Bind RanGDP  
Yuri Kim

Free Afternoon to Explore!

Milwaukee has so much to offer, so this afternoon is for you to explore the area. You’ll be surprised by how much “outdoors” there is here, from hiking trails in Veterans Park, McKinley and Bradford beaches on freshwater Lake Michigan, or golfing. Engage with local culture at the Harley-Davidson Museum, Discovery World, or a Brewers baseball game.

Obviously, Milwaukee is steeped in beer history. Check out the MillerCoors factory tour or one of the dozens of craft breweries that have sprung up. You’ll find great bars, restaurants, and more in Milwaukee’s booming entertainment districts:

RiverWalk  
Historic Third Ward  
Old World Third Street

6:00 – 8:00 p.m.  
Evening Reception and Poster Session B – Regency AD  
See above for poster details.
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<thead>
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<td>8:00 – 10:00 a.m.</td>
<td>Concurrent Scientific Session</td>
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<td>9: Host, Vector, and Pathogen Interactions I</td>
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<td>10: Pathogenesis/Pathophysiology/Cell Biology II</td>
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<td>Session Chair: Minal Mulye</td>
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<td>Session Chair: Elizabeth Case</td>
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<td>8:00 a.m.</td>
<td>Abstract #119: Identification and Characterization of a Novel Adhering</td>
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<td>Receptor for Spotted Fever Group</td>
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<td><em>Rickettsiae</em></td>
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<td>8:15 a.m.</td>
<td>Abstract #120: Crossbreeding <em>Amblyomma maculatum</em> Group Tick</td>
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<td>Populations from Distinct Geographical Regions within the United States</td>
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<td>Michelle Allerdice</td>
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<td>8:30 a.m.</td>
<td>Abstract #121: Dynamics of <em>R. rickettsii</em> Transmission at the</td>
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<td>Tick/Host Interface</td>
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<td>Michael Levin</td>
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<td>8:45 a.m.</td>
<td>Abstract #122: Comparative Virulence of Diverse <em>Coxiella burnetii</em></td>
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<td>Strains in Vivo</td>
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<td>Carrie Long</td>
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<td>9:00 a.m.</td>
<td>Abstract #123: Investigating the Role of Metabolic Genes in Intracellular</td>
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<td>Replication of <em>Coxiella burnetii</em></td>
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<td>Mebratu A. Bitew</td>
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<td>9:15 a.m.</td>
<td>Abstract #124: Altering Lipid Droplet Homeostasis Affects *Coxiella</td>
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<td><em>burnetii</em> Intracellular Growth</td>
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<td>Minal Mulye</td>
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<td>9:30 a.m.</td>
<td>Abstract #125: Molecular Tuning of Tick Cell Signaling by a Rickettsial</td>
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<td>Pathogen</td>
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<td>Girish Neelakanta</td>
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<td>9:30 a.m.</td>
<td>Abstract #126: Investigating the Role of Metabolic Genes in Intracellular</td>
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<td>Replication of <em>Coxiella burnetii</em></td>
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<td>9:30 a.m.</td>
<td>Abstract #127: <em>E. chaffeensis</em> TRP120 Involved in Activation of</td>
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<td>Conserved SHH Pathway</td>
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<td>Shubhajit Mitra</td>
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<td>9:30 a.m.</td>
<td>Abstract #128: <em>Ehrlichia chaffeensis</em> Incorporates Host Membrane</td>
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<td>Components</td>
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<td>Mingqun Lin</td>
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<td>9:30 a.m.</td>
<td>Abstract #129: Outer Membrane Protein B Enables <em>Rickettsia parkeri</em></td>
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<td>to Evade Antibacterial Autophagy</td>
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<td>Patrik Engstrom</td>
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<td>9:30 a.m.</td>
<td>Abstract #130: Distinct Ank Repeats Mediate <em>Anaplasma phagocytophilum</em></td>
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<td>AnkA-DNA Binding and Repression of Host CYBB Expression</td>
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<td>9:30 a.m.</td>
<td>Abstract #131: Identification of a Notch Activation Motif in</td>
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<td><em>Ehrlichia chaffeensis</em> TRP120 Effector</td>
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<td>LaNisha Patterson</td>
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<td>9:30 a.m.</td>
<td>Abstract #132: Distinct Bacterial Stages during the Intracellular</td>
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<td>Infection Cycle of <em>Orientia tsutsugamushi</em></td>
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<td>Jeanne Salje</td>
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<td>9:30 a.m.</td>
<td>Abstract #133: Rickettsia Exploit the Inflammasome to Avoid the</td>
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<td>Killing Effects of Type I Interferon</td>
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<td>Thomas Burke</td>
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9:45 a.m.  Abstract #126: Anaplasma phagocytophilum Asp14 Exploits Host Cell Surface Protein Disulfide Isomerase Activity to Promote Infection  ~ Ryan Green

10:00 – 10:30 a.m.
Refreshment Break – Regency AD

10:30 – 12:00 p.m.
Concurrent Scientific Session

11: Host, Vector, Pathogen Interactions II
Session Chair: Monika Danchenko

12: Infection, Diagnosis & Treatment
Session Chair: Cesar Lugo

Regency Ballroom B

10:30 a.m.  Abstract #135: Transkingdom Intercommunication at the Vector-Pathogen-Host Interface
Joao Pedra

10:45 a.m.  Abstract #136: Dissemination of Orientia tsutsugamushi and Immunological Responses in the Humanized DRAGA Mouse
Wei-Mei Ching

11:00 a.m.  Abstract #137: A Pathogen and a Non-pathogen SFG Rickettsia Trigger Differential Metabolic Signatures in Macrophage-like Cells
Ryan Green

11:15 a.m.  Abstract #138: Dispersal of the Trans-Golgi Network by the Effector RARP2 of Rickettsia rickettsii Inhibits Host Cell Protein Transport to the Cell Surface
Karin Aistleitner

Regency Ballroom C

10:30 a.m.  Abstract #140: Phenotypic Characterization and Comparison among Geographically Different Isolates of Rickettsia rickettsii
Maria Galletti

10:45 a.m.  Abstract #141: Australian SFG Rickettsiae in Ticks from Queensland, North-eastern Australia
Stephen Graves

11:00 a.m.  Abstract #142: Q Fever: A Continued Threat to the Military. Can Antibiotic Prophylaxis Reduce the Severity of Disease?
Katherine Clay

11:15 a.m.  Abstract #143: Development of a Serologic Tool for Differential Diagnosis of Rickettsiosis
Cesar Lugo

11:30 a.m.  Abstract #144: Intervention against Tick-borne Diseases in a Mayan Community
Karla Dzul-Rosado

11:45 a.m.  Abstract #145: Novel Adjuvant Systems Elicit Unique and Protective Cellular and Humoral Immune Responses in Q Fever Challenge Model Using a Polyvalent C. burnetii Vaccine
Adrienne Gilkes

29th Meeting of the American Society for Rickettsiology is Adjourned
Abstracts #1-61

Genetic Transformation and Cellular Microbiology in *Rickettsia*

Poster Session A: Epidemiology, Ecology, Infection, Diagnosis & Treatment

Axenic Culture and Genetic Transformation in *Coxiella*

*Anaplasma* and *Ehrlichia* – Vector-transmitted, Vacuolar Bacterial Genetic Manipulations

Genomics, Transcriptomics, Proteomics

Epidemiology and Ecology

Funding for this conference was made possible [in part] by R13 AI126727-01 from the 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. Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.
Abstract #1
Beating the Odds: Challenges and Perspectives in Random Mutagenesis of Rickettsiales
Ulrike G. Munderloh1*, Michael J. Herron1, Roderick F. Felsheim1, Nicole Y. Burkhardt1, Patricia A. Valdes2, Curtis M. Nelson1, and Timothy J. Kurtti1;1 Department of Entomology, University of Minnesota;2 Department of Pathology, University of Texas Medical Branch

Mutational analysis is an efficient approach to identifying microbial gene function, which can further be exploited to identify live attenuated mutants to serve as vaccine candidates. Until recently, lack of an effective tool for Rickettsiales yielding reproducible results has created an obstacle to functional genomics, and surrogate systems, e.g., ectopic gene expression and analysis in E. coli, may not provide accurate answers. We focused on generation of mutants via random mutagenesis as a potentially high-throughput method. We chose the Himar1 transposase system that results in random insertion into AT dinucleotide sites, which are abundant in Rickettsiales, and does not require contributing host factors. To drive expression, we use the Anaplasma marginale tr promoter, and the clinically irrelevant antibiotic spectinomycin for selection. To expand the repertoire of non-controversial selection markers, we successfully implemented non-antibiotic selection using an herbicide resistance gene. These constructs function reasonably well in Anaplasma, Ehrlichia and Rickettsia. We describe protocols developed in our laboratory, and discuss what likely makes them successful. To facilitate replacing the transposon with other sequences in specific mutants, including restoration of gene function using an intact copy, we added mismatched lox sites flanking the transposon. This allows swapping in desired sequences using recombinase-mediated cassette exchange (RMCE). We have designed an R. typhi-multi-epitope array that, when expressed in Rickettsia parkeri used to infect rodents, induces an antibody-response to R. typhi-specific antigens, as well as R. parkeri itself. This approach opens new avenues to create live, attenuated vaccine platform rickettsial strains that are able to express multivalent antigenic epitopes of intracellular pathogens in the intracellular environment, directing an appropriate immune response.

Abstract #2
Challenges (and Some Successes) in Spotted Fever Group Rickettsia Genetic Manipulation
Sean P Riley*, Danial A Garza, Abigail I Fish, Pedro Curto, Isaura Simões, Juan J Martinez
Vector-borne Disease Laboratories, Department of Pathobiological Sciences
Louisiana State University School of Veterinary Medicine

The herculean task of developing methods for genetic manipulation of Rickettsia species was undertaken by a community of extremely dedicated scientists. First and foremost, it is important to acknowledge the trials and tribulations of past researchers in developing a series of genetic tools that allow contemporary scientists to ask questions about the biology of Rickettsia species. The dedication of the “Rickettsia genetic manipulation pioneers” have impacted the entire field, and will undoubtedly contribute to more efficient inquiry, a better understanding of Rickettsia biology, and improved treatment strategies. From initial attempts at introducing exogenous DNA into Rickettsia species to mutagenesis of the Rickettsia genome, we have collectively overcome many of the challenges of genetic manipulation. The community now possesses viable (but extremely challenging) genetic tools, including: replicative plasmids, transposons, homologous recombination, fluorescent protein-encoding genes, and antibiotic selectable markers. It is the responsibility of contemporary researchers to further optimize these genetic tools and to employ our resources to the most productive ends. Our laboratory has employed genetically engineered plasmids to examine the pathophysiology of Spotted Fever Group Rickettsia in small animal models of infection. In addition, we have developed cell sorter-based methods for isolating individual clones of transformed bacteria. Among these successes, we have encountered a series of challenges, and I will describe our efforts to increase efficiency of rickettsial transformation. Finally, one of the greatest values of genetically tractable Rickettsia is that the community will be able to approach our research in new and exciting ways. I will describe the types of forward genetic approaches that can be employed for unbiased inquiry into the genetic basis of Rickettsia pathogenesis.
**Abstract #3**

Forward genetic analysis identifies factors crucial for *Rickettsia parkeri* cell-to-cell spread

Natasha Kafai¹, Arianna Scricco², Matthew Welch³, Rebecca Lamason*²

¹Medical Scientist Training Program, Washington University in St. Louis School of Medicine; ²Department of Biology, Massachusetts Institute of Technology; ³Department of Molecular and Cell Biology, University of California, Berkeley

Rickettsia parkeri is a member of the spotted fever group (SFG) of rickettsiae that causes an eschar-associated rickettsiosis. Like other members of the SFG, *R. parkeri* invades non-phagocytic cells, enters the cytosol and hijacks the host actin cytoskeleton to polymerize actin tails and drive motility. Motile *R. parkeri* move to a host cell-cell junction and then lose their actin tails before entering into a short plasma membrane protrusion that is engulfed into the recipient cell and resolves into a double-membrane vacuole. The bacterium then lyses this vacuole to enter the cytosol and begin the process again. Despite the importance of cell-to-cell spread to virulence, little is known about which bacterial and host proteins are required during this process. One barrier to discovery has been the limited tools available for genetic manipulation of Rickettsia. To overcome this, we sought to identify important rickettsial factors that promote spread by conducting a forward genetic screen using the Himar1 mariner-based transposon system to generate mutants with a small plaque phenotype. So far, we have identified over 100 mutants targeting genes predicted to function in a variety of pathways, including bacterial replication, metabolism, effector secretion, as well as those known to target host pathways. By combining this forward genetic screen with multidisciplinary approaches, we have begun to reveal the molecular details of the spreading process. For example, we discovered that the secreted effector Sca4 regulates host intercellular tension to promote spread, and current work in the lab is elucidating the role of additional effectors. We have also complemented this work using reverse genetic screens in the host cell to reveal which pathways may regulate pathogen spread. By leveraging these different screening modalities, our work will reveal key insights into how rickettsial factors reprogram host machinery during cell-to-cell spread and advance our understanding of how obligate intracellular pathogens interact with their host.

**Abstract #4**

*Dokkaebi* transposon mutagenesis identifies genes important for *R.conorii* life-cycle

Hwan Keun Kim¹,²* and Olaf Schneewind¹,²

¹Howard Taylor Ricketts Laboratory, Argonne National Laboratory, Lemont, Illinois, USA
²Department of Microbiology, University of Chicago, Chicago, Illinois, USA.

*Rickettsia conorii* is a Gram-negative obligate intracellular pathogen that has evolved to colonize ticks (*Rhipicephalus sanguineus*) and cause tick-borne Mediterranean Spotted Fever in humans. *R. conorii*, along with other *Rickettsia* species, has undergone reductive genome evolution optimized for parasitic life cycle. In this lifestyle, the viability of *R. conorii* depends on their ability to attach and invade the host cells, survive and replicate within the cytoplasm and evade the host immune system. Recent advances in molecular genetic tools in the *Rickettsia* field allowed investigators to begin establishing correlations between rickettsial genes and their functions. However, genes required for *R. conorii* survival and pathogenesis in ticks and humans remain largely unknown. Here, we demonstrate an *in vitro* insertional mutagenesis system in which purified Tn5-transposases complexed with the *dokkaebi* mini-transposons allow facile isolation of chloramphenicol-resistant *R. conorii* variants. PCR amplification and sequencing verified random insertion sites of the *dokkaebi* mini-transposon within *R. conorii* genome. Endowed with this technology, we identified *R. conorii* variants with defects in the parasitic life cycle in tissue culture system and the pathogenesis of Mediterranean Spotted Fever in mouse infection model. In one of the isolated *R. conorii* variants (HK2), the *dokkaebi* transposon disrupted a conserved gene cluster predicted to be involved in the biosynthesis of UDP-2-acetamido-2,6-dideoxy-L-glucose, a structural constituent of lipopolysaccharide that may serve as an epitope for serological diagnosis of human rickettsioses in the Weil-Felix reaction. Our results show that the HK2 mutant displayed atypical lipopolysaccharide on bacterial surface, eliciting unique immune responses different from that of the wild-type infection in mice.
**Abstract #5**

Genetic diversity of *Bartonella spp.* in wild mammals and ectoparasites in Brazilian Pantanal

Marcos Rogério André¹, Keyla Carstens Marques de Sousa¹, Renan Bressianini do Amaral¹, Heitor Miraglia Herrera², Filipe Martins Santos², Gabriel Carvalho Macedo², Pedro de Andrade Pinto³, Darci Moraes Barros-Battesti¹, Rosangela Zacarias Machado¹

¹Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil
²Universidade Católica Dom Bosco, Campo Grande, MS, Brasil, ³Universidade Federal da Paraíba, Laboratório de Ecologia Animal, Rio Tinto, PB, Brasil

The present work aimed to investigate the genetic diversity of Bartonella in mammals and ectoparasites in Pantanal wetland, Brazil. For this purpose, 31 Nasua nasua, 78 Cerdocyon thous, seven Leopardus pardalis, 110 wild rodents, 30 marsupials, and 42 dogs were sampled. DNA samples were submitted to a quantitative real-time PCR assay (qPCR). Positive samples in qPCR were submitted to conventional PCR assays targeting other five protein-coding genes. Thirty-five wild rodents and three Polygenis (P.) bohlsi bohlsi flea pools showed positive results in qPCR for *Bartonella* spp. Thirty-seven out of 38 positive samples in qPCR were also positive in cPCR assays based on ftsZ gene, nine in nuoG-cPCR, and six in gltA-cPCR. Concatenated phylogenetic analyses showed that two main genotypes circulate in rodents and ectoparasites in the studied region. While one of them was closely related to Bartonella spp. previously detected in Cricetidae rodents from North America and Brazil were detected, the other one was related to Bartonella alsatica, Bartonella pachyuromydis, Bartonella birtlesii, Bartonella acomydis, Bartonella silvatica and Bartonella callosciuri. These results showed that at least two Bartonella genotypes circulate among wild rodents. Additionally, the present study suggests that Polygenis (P.) bohlsi bohlsi fleas could act as possible Bartonella vectors among rodents in Pantanal wetland, Brazil.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

**Abstract #6**

Evidence and molecular characterization of *Bartonella* spp. in bats in Brazil

Marcos Rogério André¹; Priscila Ikeda¹; Meire Seki²; Adriano Carrasco²; Lucénia Rudiak²; João Miranda²; Sabrina Gonçalves²; Estevam Hoppe²; Ana Cláudia Albuquerque³; Marta Teixeira²; Camila Passos¹; Karin Werther¹; Rosangela Zacarias Machado¹

¹Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil
²Universidade Estadual do Centro-Oeste, Campus CEDETEG, Guaraí, Paraná, Brazil
³Universidade Estadual Paulista (Unesp), Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil
⁴Instituto de Ciências Biomédicas, Universidade de São Paulo (USP), São Paulo, Brazil

The order Chiroptera is considered the second largest group of mammals in the world, hosting important zoonotic virus and bacteria. *Bartonella* are bacteria that parasite different mammals species including humans, causing different clinical manifestations. The present work aimed investigating the occurrence and assessing the phylogenetic positioning of *Bartonella* spp. in neotropical bats sampled from Brazil. Between December 2015 and April 2016, 325 blood and/or tissues samples were collected from 162 bats comprising 19 different species sampled in five states of Brazil. Out of 322 bat samples collected, while 17 (5.28%) were positive to qPCR for *Bartonella* spp. based on *nuoG* gene. Seven sequences were obtained for Bartonella (*nuoG* [n=3], *gltA* [n=2], *rpoB* [n=1], *ftsZ* [n=1]). In the phylogenetic analysis, the *Bartonella* sequences clustered with *Bartonella* genotypes detected in bats sampled in Latin America countries. The present work showed the first evidence of circulation of *Bartonella* spp. among bats in Brazil. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)
According to the CDC, Q fever is a disease caused by the bacteria Coxiella burnetii. People get infected by breathing in dust contaminated by infected animal feces, urine, milk, and birth products containing Coxiella burnetii. The agent naturally infects over 40 species of ticks found in five continents. A study of the vector pools in Georgia revealed that 33% of sample pools were positive for Rickettsia spp. This abstract is focused on studying Q fever cases and relative biosafety precautions taken in NCDC laboratory network in Georgia. In Poti, the first confirmed Q fever case was registered in 2014. Initially the case was classified as a measles by the clinical doctor. Under Cooperative Biological Engagement Program (CBEP), regional LSS-s staff are trained in BSL2 biosafety practices and techniques including engineering and administrative controls, containment principles, lab practices/procedures, and safety equipment. The following biosafety approaches were used for sample collection, blood sample centrifugation and sera preparation, packaging and transfer of serum samples to Lugar Center for further diagnostics. Based on serologic tests, diagnosis of measles has been reversed. Serum samples were analyzed using ELISA method for detection Ig M and Ig G for Q fever. Test was positive for Ig M and the case was confirmed to be Q fever. Additionally 4 cases were confirmed in Georgia in 2014(Tbilisi, Mtskheta-mtianeti, Kakheti, Kvemo Kartli regions). In 2014-2017, 5 samples were taken by Gori LSS. In 2017, 1 of them was confirmed by detection of Ig M. NCDC local laboratories play an important role in the diagnosis and management of Q fever and other rickettsioses in Georgia.

The genus *Bartonella* consists of pleomorphic Gram-negative bacteria that induce long-lasting intraerythrocytic and endotheliotropic infections within the vasculature of dogs, humans and other animals. More than 10 *Bartonella* species have been reported to infect dogs worldwide. In North America, *B. henselae* (*Bh*), *B. koehlerae* (*Bk*) or *B. vinsonii* subsp. *berkhoffii* (*Bvb*) have been isolated from dogs with endocarditis, vasoproliferative pathology, vasculitis, myocarditis, polyarthritis, granulomatous lymphadenitis and hepatitis, epistaxis, and neurologic symptoms. Because of the seemingly wide spectrum of clinical presentations reported in dogs, defining when a clinician should include bartonellosis among other differential diagnoses remains unclear. To identify potential hematological abnormalities in dogs with serological evidence of *Bartonella* exposure, a serosurvey was conducted using whole cell protein lysates from each of the three clinically relevant *Bartonella* species (*Bh, Bk, and Bvb*) as ELISA antigens. Three separate ELISA assays were used to test archived dog sera (n = 5957) submitted to IDEXX Reference Laboratories from across the United States between May and August 2016. Chi-squared analyses were performed for each individual hematological parameter relative to the reactivity of dogs to any one of three *Bartonella* lysate ELISAs. 362 dogs were seroreactive (testing reactive at least twice out of three replicates on each of three ELISAs) to at least one of three *Bartonella* species. Complete blood counts were available for 4099 dogs, of which 243 (5.92%) were *Bartonella* seroreactive. Anemia, neutropenia, and eosinopenia (corresponding p-

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<th>Hematocrit (%)</th>
<th>Neutrophils (cells/uL)</th>
<th>Eosinophils (cells/uL)</th>
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<tr>
<td>Upper Specification (normal range) =</td>
<td>56.5%</td>
<td>12670</td>
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<tr>
<td>Lower Specification (normal range) =</td>
<td>38.3%</td>
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<td>highest value =</td>
<td>38.1%</td>
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<tr>
<td>lowest value =</td>
<td>21.8%</td>
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<tr>
<td>average =</td>
<td>33.1%</td>
<td>2510</td>
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<tr>
<td>median =</td>
<td>34.0%</td>
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<td>1 standard deviation =</td>
<td>4.2%</td>
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<td>dogs with below normal range values, n =</td>
<td>34</td>
<td>10</td>
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Table of Descriptive Statistics for Hematocrit, Neutrophil & Eosinophil Cell Counts
For Dogs with Values Below Normal Reference Ranges
values of 0.0465, 0.0029, and 0.0126, respectively) were associated with Bartonella spp. seroreactivity by ELISA. Reticulocyte counts for 28/34 anemic dogs (82%) were within normal range suggesting that most anemias were non-regenerative; whereas auto-agglutination was reported in 11 dogs and spherocytes with an accompanying strong regenerative response in one dog. Seroprevalence was low, consistent with a recent report using IFA testing and the same Bartonella species/subspecies. Most seroreactive dogs had variable or no hematological abnormalities. The medical relevance of the hematological abnormalities in the dogs from this study could not be determined, as the general health status, reason for submitting a complete blood count (annual examination, blood donor screening, or illness), prior administration of antibiotics or immunosuppressive drugs, and infection status are unknown. Based on the findings from our study, combinations of anemia, neutropenia and eosinopenia may represent hematological indicators of bartonellosis in sick dogs that may warrant testing for Bartonella.

Abstract #9
Molecular detection of Bartonella in South American Fur Seals (Arctocephalus australis) from Chilean Patagonia
Pedro Bittencourt¹, Mauricio Seguel², Ananda Müller³, Sandra Peréz³; Paulina Sepúlveda³; Ricardo Gutíérrez⁴, Yaarit Nachum-Biala¹, Shimon Harrus⁴
¹Escuela de Medicina Veterinaria, Facultad de Ciencias, Universidad Mayor Sede Temuco, Chile ²Department of Pathology, College of Veterinary Medicine, University of Georgia, 501 DW Brooks, Athens, Georgia 30602, USA ³Instituto de Ciencias Clínicas Veterinarias, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile. ⁴Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

The aim of this study was to perform a molecular survey of Bartonella spp. in free-ranging South American Fur Seals (Arctocephalus australis) from Guano Island, Chilean Patagonia. A total of 69 blood samples taken from 11 lactating South American Fur Seals and 58 pups from on Guano Island, southern Chile. DNA extracted from the samples was submitted to Real Time PCR (qPCR) for Bartonella spp. 16S–23S internal transcribed spacer (ITS). Positive samples on ITS qPCR screening were further submitted to four qPCR assays targeting Bartonella spp.: 16srRNA, rpoB, gltA and SsrA gene fragments. All positive qPCR products were purified and subsequently sequenced for speciation. Two out of 11 (18%) Fur Seal adults were positive for Bartonella spp. ITS gene, and none of the 58 pups was positive. ITS fragments from sequenced Bartonella spp. showed that sample #H1 (142bp sequence, not deposited on GenBank) had 100-100% identity with B. henselae isolates and sample #H2 (124bp sequence, not deposited on GenBank) shared 100-99% identity to B. clarridgeiae. Of the two ITS qPCR-positive samples, none showed positive results in cPCR assays based on gltA, SsrA and 16s rRNA genes. Sample #H2 was positive in the rpoB gene (169bp sequence, not deposited on GenBank) and shared 100% identity with B. Rochalimae. This is the first report of Bartonella positivity in Arctocephalus australis. Little is known about geographic distribution and potential reservoir of Bartonella species infecting marine mammals. Bartonella henselae was detected in harbor porpoises (Phocoena phocoena), captive and free ranging, hunter-harvested beluga whales (Delphinapterus leucas), harbor seals (Phoca vitulina) and captive cetaceans. Further studies are necessary to fully characterize which Bartonella species are present in South American Fur Seals and it’s potential role in the ecology of those organisms.

Abstract #10
Molecular detection and characterization of Bartonella spp. and Rickettsia spp. in ectoparasites from bats in Brazil
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The family Streblidae comprises a monophyletic group of Hippoboscoidea hematophagous dipterans that parasitizes bats. Although the occurrence of Bartonella spp. and Rickettsia spp. has been reported in bats sampled in Europe, Africa, Asia, and Central and South America, few are the reports on Bartonella and Rickettsia in Hippoboscoidea flies. While mites belonging to Spinturnicidae family are ectoparasites found only in bats, those belonging to Macronyssidae family also parasitize other mammal species. The present work aimed to investigate the occurrence and assessing the phylogenetic
Rickettsioses are among the important vector-borne pathogens that affect humans and animals. Infection with *Rickettsia* spp. and *Coxiella burnetii* may be unnoticed in animals, but are usually highly pathogenic to humans. The tropical climate of the Philippines highly favors the life cycle of ticks *Rhipicephalus* (*Boophilus*) *microplus* and *R. sanguineus* which primarily affects cattle and dogs, respectively. These ticks are prevalent in the country throughout the year, which greatly contributes to the spread of various tick-borne pathogens (TBPs). However, little is known about the occurrence of *Rickettsia* spp. and *C. burnetii* in the Philippines. Our group has been investigating the occurrence of various TBPs affecting ruminants and dogs in selected areas of Luzon, the largest island of the Philippines, through nested PCR (nPCR). Here we report the results for *Rickettsia* spp. and *C. burnetii* detection. Blood samples were collected from a total of 250 cattle and water buffaloes, 160 goats and sheep, and 258 dogs. Tick samples were also collected if present in the animals. nPCR targeting *glt*A gene of *Rickettsia* spp. was performed on DNA from ruminants and dogs, while nPCR targeting *com1* gene was performed for DNA samples from large ruminants. Positive bands for *Rickettsia* spp. were observed in blood samples from 6 (2.3%) dogs. Interestingly, some of these dogs also tested positive for other TBPs, and there are some that did not show any clinical signs at the time of sample collection. No band for *C. burnetii* was detected in any of the tested samples. Sequence analysis of positive amplicons revealed high identity with *Rickettsia japonica*. To our knowledge, this is the first molecular evidence of *Rickettsia* spp. in dogs in the Philippines, which should raise awareness due to potential public health implications.

**Abstract #11**

**Molecular detection of *Rickettsia* spp. and *Coxiella burnetii* in ruminants and dogs from selected areas of Luzon Island, Philippines**

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Rickettsioses are among the important vector-borne pathogens that affect humans and animals. Infection with *Rickettsia* spp. and *Coxiella burnetii* may be unnoticed in animals, but are usually highly pathogenic to humans. The tropical climate of the Philippines highly favors the life cycle of ticks *Rhipicephalus* (*Boophilus*) *microplus* and *R. sanguineus* which primarily affects cattle and dogs, respectively. These ticks are prevalent in the country throughout the year, which greatly contributes to the spread of various tick-borne pathogens (TBPs). However, little is known about the occurrence of *Rickettsia* spp. and *C. burnetii* in the Philippines. Our group has been investigating the occurrence of various TBPs affecting ruminants and dogs in selected areas of Luzon, the largest island of the Philippines, through nested PCR (nPCR). Here we report the results for *Rickettsia* spp. and *C. burnetii* detection. Blood samples were collected from a total of 250 cattle and water buffaloes, 160 goats and sheep, and 258 dogs. Tick samples were also collected if present in the animals. nPCR targeting *glt*A gene of *Rickettsia* spp. was performed on DNA from ruminants and dogs, while nPCR targeting *com1* gene was performed for DNA samples from large ruminants. Positive bands for *Rickettsia* spp. were observed in blood samples from 6 (2.3%) dogs. Interestingly, some of these dogs also tested positive for other TBPs, and there are some that did not show any clinical signs at the time of sample collection. No band for *C. burnetii* was detected in any of the tested samples. Sequence analysis of positive amplicons revealed high identity with *Rickettsia japonica*. To our knowledge, this is the first molecular evidence of *Rickettsia* spp. in dogs in the Philippines, which should raise awareness due to potential public health implications.
Abstract #12
Filling in the Gaps: A Survey of Ticks (Acari: Ixodidae) across the Northern Counties of Missouri
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The diversity of tick species occurring in Missouri and their geographical distributions are not well established. Although several tick studies have been published from Missouri, these papers do not always provide county-level resolution and are generally focused on a few species. In 2009, a large acarological survey of ticks was conducted in a one week period across much of the state south of Columbia and the Missouri River. This project provided new data on the spatial distribution of *Amblyomma americanum*, as the species constituted over 99% of the ticks collected in this survey. Since then, the data have been utilized for the creation of county-level distribution maps and models of suitable habitat in the United States for this species. However, the omission of the northern counties of Missouri from inclusion in the datasets is problematic as contemporaneous and subsequent studies have shown human cases of tick-borne viral and rickettsial diseases and tick collections from the northern region. In the present survey, we sought to document occurrence in areas where *Amblyomma americanum* was thought to exist, but where actual scientific collections had not been recorded. We targeted conservation areas in northern counties and flagged vegetation at each county site over nine days in late summer (30 July–9 August 2017). Sites in thirty-four counties were sampled, resulting in a total collection of 927 ticks. Three important human-biting species were identified in this survey. *Amblyomma americanum* larvae, nymphs and adults were found at 85% of the sites, while *Dermacentor variabilis* adults were found at 79% of the locations. *Amblyomma maculatum* adults were found at 12% of the sites. This new survey, combined with data from other laboratory and field studies, will provide baseline information on the distribution of ticks and their pathogens across the state as we initiate integrated ecological surveillance.

Abstract #13
Prevalence and Strain Diversity of *Anaplasma marginale* in Kansas Cattle Herds
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*Anaplasma marginale*, the causative agent of bovine anaplasmosis, is an obligate-intracellular tick-borne rickettsial pathogen that can be found worldwide and is endemic throughout the United States. Bovine anaplasmosis is conservatively estimated to cost the U.S. cattle industry >$300 million per year. Towards understanding the impact of this disease in Kansas, the goal of this study is to determine the prevalence of active *A. marginale* infections in cattle herds across the state of Kansas. The specific aims of this study are to: i) examine the anaplasmosis infection prevalence in Kansas cattle herds; ii) evaluate within-herd anaplasmosis infection prevalence; and, iii) identify actively circulating *A. marginale* strains. Analysis of cattle blood by PCR detection of the *A. marginale* major surface protein 5 (Msp5) gene revealed that anaplasmosis occurs throughout Kansas, with the majority of infected herds residing in the eastern third of the state. Examination of actively circulating *A. marginale* strains by amplification of a portion of the major surface protein 1a (Msp1a) gene, demonstrated that many *A. marginale* strains are circulating throughout Kansas, with most herds infected with multiple strains. Our results support the need for continued research efforts on bovine anaplasmosis to identify drivers of disease transmission and to evaluate impact of disease to the Kansas cattle industry. The data generated from this study will be the basis for future studies examining bovine anaplasmosis disease ecology including evaluation of vector transmission variables and efficacy of current treatment and control practices.
Abstract #14
Molecular detection of *Anaplasma phagocytophilum* in a patient and a tick collected from the patient in South Korea

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Human granulocytic anaplasmosis (HGA) is a tick-borne infectious diseases caused by an obligate intracellular bacterium *Anaplasma phagocytophilum*. In South Korea, 18~91 cases of HGA per year have been reported since 2015, but very little is known about how *A. phagocytophilum* infects and spreads. We describe a case of HGA and PCR detection of *A. phagocytophilum* from patient’s specimen and biting tick. Tick was morphologically identified under a microscope and molecular biologically targeting 16S rRNA gene. As a results, the collected tick from patient was confirmed as *Ixodes nipponensis*, using morphological and molecular biological methods. *A. phagocytophilum* was identified from the tick and patient's blood and *A. phagocytophilum* was isolated from inoculation of the patient’s blood into HL-60 cell. The PCR sequences for 16S rRNA, ankA and msp2 were 99% similar to the other Korea *A. phagocytophilum* isolates. Our results suggest that *I. nipponensis* could act as Anaplasmosis vector in South Korea. We are currently undertaking further studies to elucidate the complete gene sequences for Korea *A. phagocytophilum* isolates. This work was supported by Korea Centers for Disease Control and Prevention (2017-NIS2002-00)

Abstract #15
Molecular identification of *Anaplasma phagocytophilum* infection in Holstein cattle in the Republic of Korea

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Background: Global warming has increased the incidence and risk of tick-borne diseases in domestic animals and humans in the Republic of Korea (ROK). In this study, we investigated the prevalence of *Anaplasma phagocytophilum* in Holstein cattle (*n* = 214) in the ROK using specific PCR assays. Methods: Between 2016 and 2017, blood samples were taken from the jugular vein of asymptomatic Holstein cattle (a total of 214 animals) from 3 different regions in the ROK. Genomic DNA was extracted from a 100-µL sample of whole blood, using the DNAeasy Blood Kit (QIAgen, Hilden, Germany) were used for *A. phagocytophilum* detection. PCR products were purified using the Accupower PCR Purification Kit (Bioneer, Daejeon, Korea) and was then used for direct sequencing (Bioneer). A phylogenetic tree was constructed based on nucleotide alignments using the neighbor-joining method. Bootstrap analysis was conducted with 1000 replicates using MEGA version 7. Results: *A. phagocytophilum* infection was detected in only two animals (0.93%, 2/214). Our findings showed that PCR assay using the 16S rRNA gene, but not *groEL*, was suitable for detection of *A. phagocytophilum* in cattle. Phylogenetic analysis based on the 16S rRNA gene showed that *A. phagocytophilum* was divided into two clades. Clade 1 included Korean isolates, such as those from dogs, cats, Korean water deer, and ticks, while *A. phagocytophilum* identified in Holstein cattle formed clade 2. Our results suggest that there is genetic variability among isolates of *A. phagocytophilum* circulating in the ROK. Conclusions: This is the first study to report *A. phagocytophilum* infection in Holstein cattle in the ROK. As *A. phagocytophilum* has zoonotic potential, additional epidemiological studies are needed to investigate the prevalence and genetic characterization of *A. phagocytophilum* from different regions and hosts. Acknowledgement: This work was performed with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ010092), Rural Development Administration, Republic of Korea.
Abstract #16
Serologic survey of *Ehrlichia canis* infection in humans and dogs in the State of Cauca (Colombia, South America)
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*Ehrlichia canis* has a worldwide distribution and causes canine monocytotropic ehrlichiosis, but has recently been associated with human infections in Central and South America. In Colombia, several reports show high seroprevalence of *E. canis* in dogs and molecular detection of *E. canis* DNA by PCR. There are documented human clinical cases in Colombia where citoplasmic inclusions consistent with ehrlichial morulae have been visualized in peripheral blood smears or *Ehrlichia*-reactive antibodies have been detected by IFA. Thus, we investigated whether humans may be infected with *E. canis* by performing a cross-sectional study in the State of Cauca (Colombia) to determine seroprevalence and associated risk factors of *Ehrlichia* infection in humans and canines. Blood samples were randomly collected from healthy adults (n=506) and dogs (n=114) from rural areas in four municipalities. Human sera were screening by ELISA using the immunodominant and highly conserved, species specific *E. canis* TRP19 peptide. Recombinant proteins that included *E. canis* specific antigens TRP140, TRP36, and TRP19 were used to screen dog sera for *E. canis* antibodies. *E. canis* antibodies were not detected in human sera, but the majority of dogs (76%) were seropositive. This study suggest that although *E. canis* infection in dogs is highly prevalent in these municipalities, there was no evidence of zoonotic transmission to humans. Moreover, periodic epidemiological surveillance on dogs in those areas and effective control measures for the brown dog tick should be considered to reduce *E. canis* infections.

Abstract #17
BIG DATA - WORLDWIDE SNAP® 4Dx® AND SNAP® 4Dx® PLUS CLINIC-BASED SEROLOGIC SURVEY OF TICK-BORNE DISEASES IN DOGS
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Tick-borne diseases in dogs are a growing issue worldwide and increasing emphasis is being placed on studying canine populations as sentinels for tick-borne diseases in people. The aim of this study was to evaluate macrogeographic trends in major tick-borne antibody test results in pet dogs over a five-year period (2011 to 2015). The data were obtained from a comprehensive international database of SNAP 4Dx and SNAP 4Dx Plus field results and used to assess the frequency of infection with or exposure to three vector-borne disease agents in dogs: *Anaplasma* spp. (AP); *Ehrlichia* spp. (EC); and *Borrelia burgdorferi* (BB). Within the United States, data were collected from 2,526,712 samples distributed across 10,722 unique postal codes. Outside of the US (OUS), data were derived from over 585,000 samples distributed across 3,122 unique postal codes from 66 countries spanning 7 regions of the world: North America (2 countries, n=345,116); Caribbean (8 countries, n=28,008); Latin America (13 countries; n=17,587); Northern Europe (15 countries, n=51,357); Southern Europe (13 countries, n=57,446); Middle East-Africa (5 countries, n=2,255 samples); and Asia-Pacific (9 countries, n=83,720). Latin America had the highest overall positive results at 39.6%, followed by the Caribbean (28.7%), Middle East-Africa (25.9%), Northern Europe (25.1%), Asia-Pacific (12.9%), United States (11.6%), Southern Europe (10.6%), and OUS North America (3.3%). Seropositive rates by region for AP, EC, BB, along with a breakdown of the overall major co-infection rates will be reviewed. This study represents the broadest worldwide canine tick-borne disease survey reported to date utilizing data from a single test platform. Co-infection data from this multiplex test may have use as an indicator of the predominant tick vectors to which dogs are being exposed in specific regions. Overall, the global seropositive rates documented in this study support the need for continued awareness of tick-borne diseases and monitoring the risks they pose to animal and public health.
Background: Scrub typhus (ST) is a vector-borne zoonotic bacterial disease caused by the obligate intracellular bacterium Orientia tsutsugamushi (OT). Clinically, scrub typhus presents with fever and other signs and symptoms similar to that of other tropical febrile illnesses leading to difficulty in clinical diagnosis. Disease burden and epidemiological data are always helpful for formulation and implementation of disease preventive strategies, including health care policies and effective control measures. Additionally, clinicians are benefited by prioritization of appropriate laboratory investigations followed by empiric therapy. Here we studied the seasonal and geographical distribution of ST cases in the state of Karnataka, a southern state of India. Methodology: This is a hospital-based cross-sectional study, conducted at the largest tertiary care hospital in south western India in Karnataka, from June 2012 to Jan 2015. The study was approved by institutional review board. Admitted adult patients of both sexes with a fever of ≥ 3 days with clinical suspicion of ST were included in the study. History and clinical materials were collected after obtaining consent. All cases were screened for common acute febrile illness such as scrub typhus, malaria, dengue fever, leptospirosis and brucellosis. Results: A total of 1036 cases were screened of which 240 (23.2%) cases were confirmed as ST based on positive nested-PCR (56kDa gene). Male cases constituted 132 (55%) and female cases 108 (45%). Median age of males and females was 38 and 40 years respectively. Significant proportion (163, 67.9%) of the cases were agriculturist by occupation. Geographically, ST cases were seen from 9 of the 14 districts from where febrile cases were included in the study period. Davanagere (40.4%) and Haveri (30.0%) had highest ST positive cases. These districts are a part of main inland plains of Karnataka from where collectively 83.3% of all the ST cases were seen. Mountainous inland districts and coastal districts positivity for ST was 11.3% and 5.4% respectively, (p<0.001). A single case was observed from Kerala state. Seasonally, 82.5% of the cases were seen in the monsoon and post monsoon months and no cases during the summer (dry) months. Conclusion: ST is a common cause of acute febrile illness requiring hospitalization in Karnataka. Agriculturists of both sexes are the common occupational group exposed for infection with OT. ST is widely prevalent in diverse geographical regions of Karnataka with highest prevalence in the districts of inland plains. Agricultural activities in these regions are mainly rain dependent. Dramatic seasonal increase in ST cases could be attributed to the increased agricultural activity during monsoon and post monsoon which brings the vectors and humans close enough leading to disease transmission. Clinicians and public health officials in the endemic regions need to be educated about this. Differential diagnosis of fever in the endemic region should include ST so that appropriate investigations are requested and appropriate empirical therapy is initiated leading to positive clinical outcomes.

Abstract #18
Seasonal and geographical distribution of scrub typhus in south western India.

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The role of cofeeding arthropods in the transmission of an emerging rickettsial pathogen

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The transitional group rickettsiae, Rickettsia felis, is a primary vector of R. felis but, R. felis has also been reported in more than 40 others species of fleas, ticks, leeches and mites species. Likewise, several field surveys have suggested transmission potential for mosquitoes that, in a laboratory study, are able to cause transient rickettsemia in mice. Analysis of cofeeding transmission indicated that infected fleas were efficient at passing rickettsiae to uninfected fleas via a blood meal; however, cofeeding transmission to different arthropod species has not been demonstrated. To analyze the potential for R. felis transmission to other arthropods, we examined cofeeding transmission between fleas and two potential arthropod vectors, the American dog tick (Dermacentor variabilis) and Anopheles quadrimaculatus mosquitoes on a vertebrate host. Results revealed that infected cat fleas transmitted R. felis to naïve D. variabilis nymphs via fleabite on a nonrickettsemic host. Transstadial transmission of R. felis from the engorged nymphs to the adult ticks was observed, with reduced prevalence of infected
adults. Similarly, *A. quadrimaculatus*, were fed with either a blood meal containing *R. felis* via a membrane feeding system or exposed to *R. felis* by cofeeding with infected cat fleas on a vertebrate host. The results showed acquisition by and persistence of *R. felis* in mosquitoes from day 1 to 12 post-feeding. Furthermore, *R. felis* from infected cat fleas also transmitted to naïve mosquitoes on a nonrikittsemic host and can persist in the mosquito for 3 days post-exposure. Our results suggest that *R. felis*-infected cat fleas can transmit the bacteria to other hematophagous vectors in the enzootic system.

**Abstract #20**

**Epidemiologic profile and clinical course of rickettsiosis cases in Southern Mexico, 2015-2017**

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In Mexico, the Rickettsia species are causal agents of diseases such as murine typhus and Rocky Mountain, which cause infections with various clinical manifestations in humans. Are transmitted through contact with infected arthropods. Wild and domestic animals are carriers of ectoparasites. In the State of Yucatan, in Southern Mexico, recent studies have confirmed diseases caused by *R. typhi* and *R. rickettsii* in humans. This study present the clinical manifestations, therapeutic approach, and principal sociodemographic characteristics that were associated with rickettsial infections. We evaluated clinical cases confirmed by Laboratory of Emerging and Reemerging Diseases for a presumptive diagnosis to Rickettsiosis in the period from 2015 to 2017. The medical history of the patients was integrated and based on the anamnesis of the patients and their family members. 206/405 patients were positive to Rickettsiosis, the prevalence was in pediatric patients with of 6 to 12 years. Frequent signs and symptoms were rash (35/106), myalgias (76/206), arthralgias (98/206) and a predominance of fever (158/206). The largest number of positive patients in women was observed. September - November the largest number of patients was received in the three years. Two fatal cases was present with hepatic and spleen enlargement was shown, lymphadenopathy and the neurological affectation. The presence of antecedents of contact with the arthropod vector in domestic dogs was readily advised by family members of patients. We cannot assure that it was intentionally investigated during the initial anamnesis in the first medical consultation. The anamnesis is an orienting process and it is relevant in the process of differential diagnosis.

**Abstract #21**

**Suburban focus of rickettsiosis in Yucatán, Mexico**

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Murine typhus, caused by *R. typhi*, is endemic in Yucatan where there are suburban settlements due to the accelerated population growth where the inhabitants present diverse health problems, highlighting the febrile syndrome where ecological and epidemiological factors could determine the prevalence of this disease. This study present the ecological and epidemiological factors associated with a suburban focus of Rickettsiosis in Yucatán. An instrument called "Epidemiological diagnosis" was applied. Indirect immunofluorescence was performed to detect IgG from *R. rickettsii* and *R. typhi*. A OR 95% confidence was made for associations of risk factors and Risk Attributable to 95% confidence to estimate excess absolute risk due to a given exposure.126 (49.2%) blood samples were taken. 44.4% (56/256) tested positive for Rickettsia tiphy and 41.3% (52/256) for *R. rickettsii*. An association was found between the presence of cats, with positive serology to *R. tiphy* (OR = 2.489), however, a reliability index of 0.783-7.907. The presence of dogs was a protective factor for Rickettsia Rickettsii serology (OR = 0.492, 95% CI 0.221-1.095). In the univariate analysis of the epidemiological and ecological variables, no association was found with the positivity of the serological samples. Rickettsiosis is present in the suburban population despite not being considered a disease with epidemiological impact. The null association of the analyzed factors and the high number of positive sera, gives us the guideline to consider that the infection comes from factors external to the epidemiological and ecological characteristics present.
**Abstract #22**

*First report of detection of Rickettsia pathogenic bacteria in trombiculid chiggers from Thailand*

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*Rickettsia* is a genus of highly pleomorphic bacteria that are causative agents of spotted fever group and typhus group rickettsioses. To date, numerous publications indicated that a wide range of ectoparasites and many species of mosquitoes are important vectors for *Rickettsia*. In the present study, we investigated the role of trombiculid larva mite (chigger) as potential vector of *Rickettsia*. We conducted entomological surveillance study of small rodents and their ectoparasites in 13 provinces covering four regions of Thailand from 2015 to 2017. A total number of 36,225 trombiculid chiggers was recovered from 883 small rodents. Majority of the chiggers belonged to *Leptotrombidium* species (32.6%, 11,811/36,225). Of 11,787 chigger samples tested (1,451 individual chiggers and 1,069 pools of 5 or 10 chiggers), 93 samples were positive by qPCRs and highly sensitive ddPCR targeting 17kDa antigen encoding gene. This corresponds to 3.7% prevalence among chigger population. Results of multilocus sequence typing analysis of five rickettsia species specific genes including 17-kDa, *gltA, ompA, ompB,* and *sca4* genes confirmed the presence of *Rickettsia* in trombiculid chiggers. Phylogenetic analyses demonstrated the presence of *R. felis*, the causative agent of flea-borne spotted fever rickettsiosis and *R. japonica* in trombiculid chiggers. Other *Rickettsia* species are discussed. *Rickettsia*-positive chiggers were found in 11 of 13 provinces with the high prevalence in the North, Northeastern and Central regions, suggesting possible wide-spread distribution of this potential Rickettsia vector in Thailand.

**Abstract #23**

*Amblyomma maculatum* group ticks in S. Arizona: an update on population range and *Rickettsia parkeri* occurrence

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Conclusively identified as a human pathogen in 2004, *Rickettsia parkeri* is a tick-borne bacterial pathogen transmitted by *Amblyomma maculatum* group ticks throughout the Americas. Within the United States, *R. parkeri* is found primarily in *Amblyomma maculatum* populations in coastal states in the southeast ranging from Texas to Virginia. However, in 2014 and 2015 2 cases of *Rickettsia parkeri* rickettsiosis from the Pajarita Wilderness in southern Arizona prompted an investigation into the range of the *Amblyomma maculatum* group tick populations in this region. Initial collection efforts in July 2016 surveyed 3 sites within the mountainous regions of Santa Cruz and Cochise Counties in southern Arizona, where 24% of the *Amblyomma maculatum* group ticks collected contained *R. parkeri* DNA. The 2016 study has established a clear public health risk in this region, and to date, there have been a total of 8 cases of *R. parkeri* rickettsiosis identified in this region. Further investigation is required to better determine the range of this tick in Arizona and the threat it poses. This present study expanded on the 2016 surveillance work, with 231 adult *A. maculatum* group ticks collected from over 10 different sites in the southern Arizona counties of Pima, Santa Cruz and Cochise in July 2017. All tick samples were individually screened for the presence of rickettsial DNA using a genus-specific *gltA* real-time assay, followed by amplification and DNA sequencing of the *ompA* gene to determine the rickettsial species present in the positive tick samples. *Rickettsia parkeri* DNA was found in 21% of these ticks and the DNA of *Candidatus “Rickettsia andeanae”* was present in 1% of the samples. Several newly identified populations of *A. maculatum* group ticks were identified in the Chiricahua, Dragoon, Huachuca, and Rincon Mountains, which yielded *R. parkeri* positive samples. With the frequent occurrence of *R. parkeri* in these *A. maculatum* group tick populations, increased education efforts and additional tick
Abstract #24

*Rickettsia asembonensis* causing febrile disease in southern Thailand, 2016-2018

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A cluster of febrile patients has been reported in Phatthalung province, southern Thailand since December 2015. Serological and real-time PCR indicated infections of *rickettsiae* and scrub typhus. To effectively control the outbreak, investigation of pathogen specific species as well as transmission cycle is essential. Patients presenting with high fever, headache, muscle pain, myalgia and skin rashes in Phatthalung province have been monitoring. Blood specimens were tested for rickettsiosis using IFA serology and *Rickettsia* 17 kDa PCR followed by DNA sequence analysis. During April 2016-February 2018, total 328 blood samples of febrile patients were received. IFA serological test revealed 7.6% (35/328) of patients had seroconversion to *Orientia tsutsugamushi*. *Rickettsia* 17 kDa and *Orientia* 56 kDa were detected in 4.0% (13/328) and 12.2% (40/328) of samples, respectively. DNA sequence analysis demonstrated flea-born *Rickettsia* species, *R. asembonensis*, *R. typhi* and *R. felis* were responsible for rickettsiosis. Survey of reservoir hosts and arthropod vectors were performed in outbreak areas to investigate for the disease transmission cycle. *Leptotrombidium* chiggers infested on captured scrub typhus seropositive rodents were found in patients’ villages. The presence of captured scrub typhus seropositive rodents infested with *Leptotrombidium sp*. Chiggers and *Ctenocephalides felis orientis* fleas infected with *R. asembonensis* in patients’ villages suggested high risk areas for infection. Health education to raise self-awareness, use of insect repellent and maintaining a clean household are also recommended in this community.

Abstract #25

Differentiation of *Rickettsia typhi*, *R. felis*, and *R. felis*-like Organisms via Restriction Fragment Length Polymorphism Analysis

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*Rickettsia typhi* and *Rickettsia felis* are flea-borne pathogens which cause murine typhus and flea-borne spotted fever, respectively. Recently, two other flea-borne rickettsiae have been discovered, *Rickettsia asembonensis* and *Candidatus Rickettsia senegalensis*. These two organisms are closely related to *Rickettsia felis*, but with enough genetic heterogeneity to propose their classification as new species. Currently, species-specific identification of detected organisms requires sequencing or probe based PCR assays. Our aim was to develop an efficient and inexpensive method to differentiate flea-borne rickettsiae via restriction fragment length polymorphism (RFLP) analysis. Outer membrane protein B (ompB) sequences of the aforementioned flea-borne rickettsiae were analyzed using DNASTAR Lasergene Core software to focus on the region amplified by the primers 120.2788 and 120.3599. Restriction enzyme digestion sites were then found, and in silico digestions of each species were compared via simulated agarose gels. After comparing hundreds of restriction enzymes, NlaIV was determined to be the most effective at creating a unique banding pattern within the area of interest. To confirm the predicted performance of NlaIV digestion, we tested the DNA of known PCR positive *Ctenocephalides felis* fleas collected from cats and opossums within Galveston, Texas. DNA from these fleas were amplified using the OmpB primer set 120.2788 and 120.3599. The PCR products were then digested with NlaIV, subjected to polyacrylamide gel electrophoresis, and visualized via ethidium bromide staining. The banding patterns were then compared to the computer-generated digestion patterns. All samples demonstrated a banding pattern consistent with the predicted pattern for the known species, as confirmed by previous sequencing. This RFLP assay was developed to be an efficient and cost effective way to screen samples for *Rickettsia typhi*, *R. felis*, and *R. felis*-like species (i.e., *Rickettsia asembonensis* and


**Abstract #26**

**Introduction of an informational website for the rickettsial disease scrub typhus**

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Increased incidence of the rickettsial disease scrub typhus (aka. mite-borne typhus) is of growing concern to medical professionals both within and outside its traditional endemic regions of the Asia-Pacific rim and southwest Pacific islands. This rising interest is due to evidence of antibiotic resistant or hypervirulent strains of *Orientia tsutsugamushi*, as well as the growing incidence of the disease both within the so called “tsutsugamushi triangle,” and within geographically disparate regions such as Africa, South America and the Arabian Peninsula. Increased attention also appears to be driven by the availability of powerful new tools such as PCR, and gene and genome sequencing, for both the detection and genetic characterization of the agents of scrub typhus. Now, the internet allows instant access to the wealth of developing data. To help organize, summarize and facilitate access to this information on scrub typhus, we have established a website aimed at introducing scientists, medical professionals and the general public to the disease, and to the tools used to detect and genetically characterize the agent. The site, “Scrub typhus and *Orientia tsutsugamushi*: geography and genotype of a vector-borne disease,” can be accessed at https://u.osu.edu/scrubtyphus/. The site brings together information on geography, diagnostic methods, antibiotic resistance, and vectors. Substantial space is allocated to the molecular genetics of *O. tsutsugamushi*, incorporating detailed information on genes that have been used to classify isolates, including the 56kD type-specific antigen, the 47kD cell surface antigen (htrA), and the 16S rRNA (rrs) genes. Information about genes that may have relevance for the analysis of potential antibiotic resistance is also provided, as are details on the genes that have been included by various research groups in their attempts to provide multi-locus sequence typing (MLST) of isolates of scrub typhus. Information about the genome sequences of 40 isolates of *Orientia* are described (including the genome sequence of *Candidatus* Orientia chuto). Historical information about scrub typhus is also presented, including historical descriptions of the disease, the history of antibiotic use to control scrub typhus, and detailed information concerning the background of isolates that have become important in research on scrub typhus. We believe this new website will prove to be a succinct, useful introduction to scrub typhus as well as a valuable resource for both new and seasoned investigators of this neglected disease.

**Abstract #27**

**Rickettsial Infections among Cats and Cat Fleas in Riverside County, California**

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Due to the re-emergence of flea-borne rickettsioses in southern California and the southern United States, and their association with *Ctenocephalides felis* (cat fleas), we aimed to investigate the presence of rickettsial infections in domestic cats (*Felis catus*) and the fleas associated with these cats in Riverside County, California. In addition, we aimed to investigate the prevalence of IgG antibodies to Spotted Fever Group Rickettsia (SFGR) and Typhus Group Rickettsia (TGR) in these cats. To this end, 30 cats and 64 pools of fleas collected from these cats were investigated for rickettsial infections. Three (10%) cats and 17 (26.6%) flea pools tested positive for rickettsial infections. PCR and DNA sequencing indicated that one cat was positive for *Rickettsia felis* infection, while 2 cats were positive for *Candidatus* Rickettsia senegalensis infections. Additionally, 12 (18.8%) flea pools were positive for *R. felis*, while 5 (7.8%) were positive for *Ca. R. senegalensis*. Interestingly, no cats or their associated fleas tested positive for *R. typhi*. Finally, eight (26.7%) sera from these cats contained SFGR antibodies, while only 1 (3.3%) contained antibodies to TGR. The detection of *R. felis* and SFGR antibodies...
and the lack of \textit{R. typhi} and TGR antibodies supports \textit{R. felis} as the main rickettsial species infecting cat fleas. The detection of \textit{Ca. R. senegalensis} in both fleas and cats also provides additional evidence that cats and their associated fleas are infected with other \textit{R. felis}-like organisms. Furthermore, the detection of \textit{R. felis} and \textit{Ca. R. senegalensis} highlights the potential risk for human infections with \textit{R. felis} or \textit{R. felis}-like organisms.

**Abstract #28**

**Febrile exanthemtatic syndrome originated by \textit{Rickettsia} in tropical regions: clinical and sociocultural differences from viral diseases**

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In tropical areas, there are several vector borne diseases that could share clinical characteristics making its diagnosis really challenging. Febrile exanthemtatic syndrome (FES), is characterized by fever, musculoskeletal pain and maculo-papular rash; that could be produced by rickettsia, but also by viral agents like dengue. However, these kind of agents have different risk factors for its transmission and subtle differences on its clinical presentation. Therefore, the objective of this work was to identify clinical differences and social determinants among pediatric patients with FES caused from rickettsia from those caused by viral agents. For this, we obtained and analyzed serum samples to identify the etiologic agent, alongside with a survey that collected data related to social determinants and knowledge from the patients related to FES caused by rickettsia and viral agents. Results were analyzed by Fischer test. 15% of the patients were positive to \textit{Rickettsia} however, we did not find clinical differences with 50% of the patients corresponding to viral diseases, and 35% that had no infectious etiology. We found several social determinants that could be associated specifically to \textit{Rickettsia} transmission mainly related to the house of the patients and its surroundings. These kind of determinants could be limited through educative interventions, allowing a better epidemiologic vigilance.

**Abstract #29**

**\textit{Rickettsiae} within the Fleas of Feral Cats in Galveston, Texas**

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Since 2012, after decades of quiescence, Galveston has experienced a resurgence in murine typhus. Once a prevalent disease, the use of DDT on rat harborages broke the cycle of rat-rat flea transmission in Galveston, as it did in the remainder of the United States. The high seroprevalence for typhus group antibodies in local opossums (66% with reactive sera) and the 7% of \textit{R. typhi}-infected \textit{Ctenocephalides felis} collected from these animals, hint at a transmission cycle involving opossums, as described elsewhere in Texas. Since \textit{Ct. felis} infests domestic animals, especially cats, we sought to determine the prevalence of \textit{R. typhi} and other fle-borne \textit{rickettsiae} within fleas collected from feral cats in Galveston. Twenty-four feral cats, trapped from all over the island as part of a spay/neuter and release program, were combed for fleas. A total of 314 fleas were collected. The fleas were grouped into 87 pools (2 – 5 fleas each) and processed for quantitative real-time PCR analysis. Twelve pools were identified as having the presence of rickettsial DNA. Subsequent PCR for the amplification of an 813 base-pair \textit{ompB} product were performed. Sequenced amplicons revealed \textit{R. typhi}, \textit{R. felis}, \textit{R. asemobonensis}, and \textit{Candidatus} \textit{R. senegalensis} in 1, 4, 1, and 6 pools, respectively. Amplification and sequencing of portions of the citrate synthase and 17-kDa antigen genes were also performed for confirmation. An estimation of the minimum infection rate of pooled flea samples revealed \textit{R. typhi} in 0.3% (95% CI, 0.1-1.5), \textit{R. felis} in 1.3% (95% CI, 0.4-3.1), \textit{R. asemobonensis} in 0.3% (95% CI, 0.1-1.5), and \textit{Candidatus} \textit{R. senegalensis} in 2.0% (95% CI, 0.8-4.0). Although \textit{R. typhi} was found among a single pool of \textit{Ct. felis} collected from these cats, the prevalence is much lower than that collected from opossums. Thus, we believe opossums are more likely linked to the reemergence of murine typhus in Galveston.
Rickettsiosis and scrub typhus are major febrile illnesses in Thailand. To effectively control the diseases, information of their transmission is essential. An active surveillance for agents causing rickettsioses and scrub typhus was conducted on Kut Island, a popular tourist destination, as well an important military position. Twelve locations on Kut Island and the shore district were surveyed using rodent trapping and arthropod collection in February 2018. A total of 44 arthropod pools were collected from dogs and captured rodents. The collected arthropods included two species of ticks (*Rhipicephalus sanguineus*, and *Haemaphysalis* sp.), one species of flea (*Ctenocephalides felis orientis*), louse (*Heterodoxus spinigerum*) and mite (*Echinolaelaps echidninus*). Using 17 kDa qPCR and species-specific qPCR, rickettsiae were detected in 23% (10/44) of arthropod specimens composed of four tick, five flea and a mite pools, respectively. *Rickettsia asemboensis* were identified in 7 of 10 *Rickettsia*-positive tick, flea, and mite pools. Total 27 rodents were captured and using IFA and *Rickettsia-Orientia* duplex PCR, 16% (6/27) of rodent sera reacted to *O. tsutsugamushi*, and 8% (3/27) were positive for rickettsiae. No *O. tsutsugamushi* DNA was detected in either arthropod or rodent samples. Our preliminary findings suggest a potential risk of human exposure to agents of rickettsiosis and scrub typhus in these areas. Local administrators and health authorities were notified to establish the community specific disease prevention strategy via a fact sheet with information providing, health education to raise self-awareness, use of insect repellent and maintaining a clean household.

**Abstract #31**

Identifying and diagnosing Bartonella infections in Morocco

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Background: The Bartonella, known since the early twentieth century, are small Gram-negative, polymorphic bacteria that infect humans as well as certain mammals. They are responsible for various neglected zoonotic pathologies namely Carrion's disease, cat scratch disease and endocarditis etc. Some of these diseases have reappeared in many parts of the world, especially in African refugee camps, homeless populations in Europe, and North America. Methods: We aim to collect 100 blood samples, for which we will conduct serology tests for different species namely Bartonella, Coxiella, Leptospirae, Brucella, and Chlamydia. Sera will be considered positive for *Bartonella* when IgG titers ≥ 1:800. Western blot and cross absorption will confirm the diagnosis of Bartonella spp. Positive sample will be diagnosed through PCR and sequencing to identify the species. Patients participating in our study should have symptoms of unexplained fever, neuroretinitis or Blood Culture–Negative Endocarditis (BCNE). Epidemiological data will be collected to guide the etiological diagnosis. The authorization of the ethics committee is under process. Results: Publicly, one study was conducted in Morocco by Boudebouch & al. shows a rate of 5.2% of BCNE caused by Bartonella quintana compared to 13% and 32.5% that represent the BCNE prevalence caused by B. quintana in Algeria and Tunisia respectively. This suggests the imminent presence of diseases related to this kind of bacteria. In fact, two cases of neuro-retinitis caused by *B. henselae* have been reported to ophthalmology departments in Rabat. Conclusions: The number of national studies related to Bartonellosis remains limited. However, the conclusions of these works are sounding the alarm on the involvement of Bartonella spp. in different human pathologies. Therefore, it is recommended to conduct additional studies at the national level to identify the incidence of Bartonella infections in the Moroccan population.
Abstract #32
Survival of Coxiella burnetii in a laboratory generated aerosol
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Coxiella burnetii, the causative agent of Q fever, has the capacity to infect a broad range of animal species, including humans. In pregnant domesticated sheep, goats and cattle infection with C. burnetii can result in abortion and still birth. These abortive events can release high concentrations of bacteria, contained within aerosols or fomites (originating from the birthing products), into the environment which can then potentially be transported long distances by the wind. C. burnetii is highly infectious by the aerosol route in humans and can result in a range of clinical manifestations from asymptomatic infection, to a self-limited flu like illness or more serious presentations such as endocarditis and chronic fatigue syndrome. C. burnetii is also believed to be able to survive for long periods in the environment due to the ability to convert to a resistant small cell variant. There have been several outbreaks of Q fever in humans associated with individuals living downwind of infected farms, including in the US, the UK and most recently in the largest reported outbreak in the Netherlands (2007 – 2010). Therefore, it is important to understand the ability of C. burnetii to survive within the environment, to understand the fundamental hazard associated with this organism and to inform future emergency outbreak response protocols. This study evaluates the viability of C. burnetii strain Nine Mile in a suspended dynamic aerosol. © Crown copyright (2018), Dstl. This material is licensed under the terms of the Open Gov’t Licence.

Abstract #33
The effect of pH on antibiotic efficacy against Coxiella burnetii in axenic media.
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Coxiella burnetii, the etiologic agent of Q Fever, replicates in an intracellular compartment with low pH. The impact of this low pH environment on antimicrobial treatment is not well understood. An in vitro system for testing antibiotic susceptibility of C. burnetii in axenic media was set up to evaluate the impact of pH on 1) C. burnetii growth and survival, and 2) the efficacy of doxycycline, hydroxychloroquine, moxifloxacin, and rifampin. The Nine Mile Phase 2 strain of C. burnetii in log phase was diluted into fresh flasks of ACCM-2 media with varying pH and antibiotic dosage. Cultures were tested by qPCR for the quantity of C. burnetii at the start of the culture and after 7 days’ incubation. Two methods were used to determine viability and growth after antibiotic exposure. In a 24 well plate, an aliquot of the culture was diluted into fresh ACCM-2, pH 4.75, and grown for 1 week. Additionally, an aliquot was placed on an ACCM-2 agar plate, pH of 4.75, and colonies counted after two weeks. The data show that doxycycline and rifampin are bacteriostatic at low doses in the ACCM-2 system. No growth was observed in cultures with as little as 0.001 ug/ml doxycycline. Changing pH from 4.75 to 5.25 improved the efficacy of doxycycline. Even at 10 ug/ml of doxycycline, double the recommended serum concentration for treatment, viable C. burnetii could be recovered from treated cultures. This indicates that even after exposure to high doses of antibiotics, C. burnetii is capable of growing and reproducing once conditions become favorable again. Hydroxychloroquine did not affect pH or growth in axenic media, but raising the pH of ACCM-2 was bacteriostatic, with C. burnetii growth ceasing above 5.75. The data suggest that the efficacy of hydroxychloroquine is primarily due to its ability to raise the pH of vacuoles.

Abstract #34
Bartonella vinsonii subsp. berkhoffii and B. henselae in dogs from Chile
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This study aimed to molecularly survey Bartonella in dogs from Chile. Quantitative real-time PCR (qPCR) for Bartonella spp. based on nuoG gene was performed in 139 blood samples taken from dogs belonging to rural localities of the Valdivia
Human Granulocytic Anaplasmosis report in Mexico. Since the first recognition in 1990 in United States, there is no evidence of this human case in Mexico despite the vector and reservoirs are reported already. We report the first clinical and molecular case of Human Granulocytic Anaplasmosis in Mexico. A 5-year-old female, presented in September, 2014 to the emergency department of a tertiary care hospital in Mexico City, with a 2-day history of fever (38.0ºC), frontal headache, productive cough, nausea and vomiting, followed by upper limb weakness that began one day prior to the admission. She lived in Estado de Mexico, in a suburban area, with her parents and one sibling. The parents denied recent travel and sick contacts. They didn’t own pets, but she had contact with a dog with ticks. The physical exam revealed intercostal retractions and bibasilar crackles, and the neurologic examination was notable for bilateral upper limb weakness (proximal 2/5 MRC, distal 1/5 MRC) and diminished stretch reflexes in the left arm. Initial laboratory study results were notable for a white blood cell count of $16.2 \times 10^9$ cells/mm$^3$ with 66% neutrophils and 21% lymphocytes, hemoglobin of 14.6 g/dL, platelets of 354 000 cells/mm$^3$, creatine kinase of 102 U/L. Liver and renal function tests were within normal limits. Lumbar puncture was performed and cerebrospinal fluid analysis revealed a white blood cell count of 20 cells/mm$^3$ with predominantly mononuclear cells (81%), red blood cell count of 286 cells/mm$^3$, protein 71 mg/dL, glucose 78 mg/dL. T-2 weighted Magnetic Resonance Imaging (MRI) showed a diffuse hyperintense signal within spinal cord from C4 to T4, without contrast enhancement. A nerve conduction velocity test revealed axonal changes and conduction block in the tibial and peroneal nerves. Electromyography showed changes compatible with bilateral L2-L4 radiculopathy. Given the unfavorable clinical course despite therapy for acute pyradiculoneuropathy. A written informed consent was obtained to their parents. Were performed in serum and CSF IFA (IgG and IgM) to *Anaplasma phagocytophilum*, and other vector-borne diseases relate. IFA (IgG) positive and PCR positive product was sequencing by 16S rRNA gene for *Anaplasma phagocytophilum* was confirmed. Treatment with doxycycline was started, and gradual improvement of lower limb strength was observed over one week, allowing for ambulation. Improvement in respiratory muscle weakness has allowed for progressive weaning of mechanical ventilation, although she still needs intermittent respiratory support with positive pressure through tracheostomy.

**Abstract #35**

First Case report of Human Granulocytic Anaplasmosis in Mexico with serological and molecular evidence

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Human Granulocytic Anaplasmosis in a tick-borne rickettsial disease caused by *Anaplasma phagocytophilum*, and transmitted by *Ixodes scapularis* tick. Since the first recognition in 1990 in United States, there is no evidence of this human case in Mexico. We report the first clinical and molecular case of Human Granulocytic Anaplasmosis report in Mexico. A 5-year-old female, presented in September, 2014 to the emergency department of a tertiary care hospital in Mexico City, with a 2-day history of fever (38.0ºC), frontal headache, productive cough, nausea and vomiting, followed by upper limb weakness that began one day prior to the admission. She lived in Estado de Mexico, in a suburban area, with her parents and one sibling. The parents denied recent travel and sick contacts. They didn’t own pets, but she had contact with a dog with ticks. The physical exam revealed intercostal retractions and bibasilar crackles, and the neurologic examination was notable for bilateral upper limb weakness (proximal 2/5 MRC, distal 1/5 MRC) and diminished stretch reflexes in the left arm. Initial laboratory study results were notable for a white blood cell count of $16.2 \times 10^9$ cells/mm$^3$ with 66% neutrophils and 21% lymphocytes, hemoglobin of 14.6 g/dL, platelets of 354 000 cells/mm$^3$, creatine kinase of 102 U/L. Liver and renal function tests were within normal limits. Lumbar puncture was performed and cerebrospinal fluid analysis revealed a white blood cell count of 20 cells/mm$^3$ with predominantly mononuclear cells (81%), red blood cell count of 286 cells/mm$^3$, protein 71 mg/dL, glucose 78 mg/dL. T-2 weighted Magnetic Resonance Imaging (MRI) showed a diffuse hyperintense signal within spinal cord from C4 to T4, without contrast enhancement. A nerve conduction velocity test revealed axonal changes and conduction block in the tibial and peroneal nerves. Electromyography showed changes compatible with bilateral L2-L4 radiculopathy. Given the unfavorable clinical course despite therapy for acute pyradiculoneuropathy. A written informed consent was obtained to their parents. Were performed in serum and CSF IFA (IgG and IgM) to *Anaplasma phagocytophilum*, and other vector-borne diseases relate. IFA (IgG) positive and PCR positive product was sequencing by 16S rRNA gene for *Anaplasma phagocytophilum* was confirmed. Treatment with doxycycline was started, and gradual improvement of lower limb strength was observed over one week, allowing for ambulation. Improvement in respiratory muscle weakness has allowed for progressive weaning of mechanical ventilation, although she still needs intermittent respiratory support with positive pressure through tracheostomy.

**Abstract #36**

The Occurrence of Human Granulocytic Anaplasmosis in South Korea during 2015-2017

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Anaplasma phagocytophilum is a gram negative bacterium and intracellular bacterial pathogen that is transmitted to mammals by ticks worldwide. In South Korea, Human Granulocytic Anaplasmosis (HGA) cases have been identified since the first report in 2014. A total of 594 clinical samples of patients with acute febrile illness were collected for laboratory diagnosis of HGA between January 2015 and May 2017. Thick peripheral blood smear, nested PCR for 16S rRNA and msp2(p44) gene and blood culture on human promyelocytic cell line HL-60 were performed to confirm HGA in this study. IgG and Ig M positivity for A. phagocytophilum was 7.91%(47/594 cases) in the patient sera and 14 of 42 cases showed seroconversion. The detection rate of 16S rRNA genes in patient blood was 3.68% (14/380 cases). Three clinical isolates were identified by genetic analysis. In the phylogenetic analysis with the isolates of other countries such as the USA, the 16S rRNA gene showed relatively high homologous (98.58–100%) and the msp2(p44) gene was very diverse (76.4-99.5%). Our results will be useful for surveillance of HGA patients in South Korea and pathogenicity studies for A. phagocytophilum in the future. This work was supported by the Korea Centers for Disease Control and Prevention (2017-NI52002-00 and 4837-301-210-13).

Abstract #37
Comprehensive Screening Of Vector-Borne Infections: Accuracy Of Rapid Tests
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Vector-borne diseases (VBD), including Lyme, ehrlichiosis, and anaplasmosis, are becoming increasingly prevalent as tick distributions are expanding. Both dogs and humans are susceptible. Comprehensive screening of VBD in dogs is diagnostically important for veterinarians and epidemiologically important for public health. Performance of the SNAP® 4Dx® Plus Test, which detects antibodies to five tick-borne pathogens and Heartworm (Dirofilaria immitis) antigen has been documented in large population studies. Another test, the VetScan® FLEX4® Rapid Test (Abaxis), was recently made available, but relative performance of the two tests has not been reported. In the present study, we compared the two tests for performance with archived canine serum and plasma samples obtained from IDEXX commercial reference laboratories and veterinary clinics. Samples were selected based on geographic distribution of canine VBD, including Lyme (n=105), A. phagocytophilum (160), A. platys (115), E. canis (154), E. ewingii (163), E. chaffeensis (151) and Heartworm (105). Infection status was established for each sample by a combination of reference methods that included necropsy (Heartworm), Western blotting (Lyme), IFA (A. phagocytophilum, E. canis) and species-specific ELISA (E. canis, E. ewingii, E. chaffeensis, A. platys). Samples were tested using the two rapid test kits following the manufacturer’s instructions. Compared to reference results, sensitivities differed substantially between the SNAP® 4Dx® Plus Test and VetScan® FLEX4® Rapid Test, with 95.5 vs. 40.9%, respectively for Lyme, 97.1 vs. 61.8% for E. canis, 98.1 vs. 59.3% for E. ewingii, 64.3 vs. 35.7% for E. chaffeensis, 84.5 vs. 12.7% for A. phagocytophilum, 83.3 vs. 33.3 for A. platys, and 94.1 vs. 88.2% for Heartworm. Both tests had specificities of at least 98%. A canine VBD screening test with a high rate of false negative results could lead to misdiagnoses and other clinically relevant issues in veterinary medicine and undermines the important role of canine VBD screening in public health epidemiology.

Abstract #38
Proteome-wide identification of novel Ehrlichia HECT-like E3 ligases
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E. chaffeensis has evolved countervailing strategies mediated in part by tandem repeat protein (TRP) effectors secreted by the type I secretion system (TISS). Insight into the functional roles of TRPs has revealed interactions with a diverse network of host proteins associated with important cellular processes and signaling pathways, including posttranslational modification (PTM) pathways. We previously reported TRP120, a multifunctional effector moonlights as a HECT E3 Ub
ligase, and is posttranslationally modified by ubiquitin (Ub) through intrinsic and host-mediated HECT ligase activity. We have determined that TRP120 has a functional C-terminal HECT Ub ligase domain involved in autoubiquitination and ubiquitination of host cell regulators (PCGF5 and FBW7) for degradation. The limited knowledge of the function of members of the *Ehrlichia* E3 family prompted us to screen in *Ehrlichia* proteome for novel E3 ligases. We propose that *Ehrlichia* may encode a large number of E3s that are capable of employing the Ub system for manipulation of host signaling pathways. In this study, to mine putative E3s from the *Ehrlichia* proteome, we first aligned the C-terminus of *Ehrlichia* proteins with several known HECT E3 ligases, and we determined that 7 *E. chaffeensis* proteins contain HECT ligase canonical conserved leucine and catalytic cysteine residues in the C-terminus. 12 proteins contain noncanonical leucine and catalytic cysteine residues in the C-terminus. Next, we examined the ubiquitination of these proteins *in vitro* using recombinant protein and a ubiquitination assay. We observed higher-molecular-mass species of *Ehrlichia* pyridine nucleotide-disulphide oxidoreductase family protein (ECH_0649) in the presence of UbcH2/3, whereas these bands were not significantly detected in the presence of other E2 enzymes after quantification analysis. This finding indicates that *Ehrlichia* pyridine nucleotide-disulphide oxidoreductase family protein may have intrinsic HECT ligase activity in the presence of UbcH2/3. The current study identifies a novel *E. chaffeensis* ubiquitin ligase, and reveals an important role of the ubiquitin pathway in effector-host interactions and pathogen-mediated host protein stability in order to promote survival in the phagocyte.

**Abstract #39**

The Case for Valid Rickettsia IgM Assays

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A 2014 paper in the American Journal of Tropical Medicine concluded “Use of IgM antibodies should be reconsidered as a basis for diagnosis and public health reporting of RMSF and other spotted fever group rickettsiae in the United States.” Although the authors were CDC and Tennessee Department of Health scientists, we suggest this warning be confined only to assays using whole-cell Rickettsia antigens and to extend this to include typhus group rickettsiosis. Such warnings should never to taken to mean that properly designed IgM assays should not be utilized in confirming acute rickettsiosis. The assumption has too often been that IFA or MIF are the only assays available, although both western immunoblot and ELISA assays have been shown to be both accurate and sensitive. Comparative results of MIF and ELISA performed in our laboratory demonstrate that the removal of LPS from the immunodominant protein antigens (native rOmp A and/or rOmpB) produces spotted fever and typhus group IgM ELISA assays that are both sensitive and specific. As removal of LPS from whole cell antigens is not realistic due to the crystalline nature of the s-layer, whole cell antigens should not be utilized for IgM antibody assays due to unacceptably high false-positive rates. Attempts were made to adsorb anti-LPS reactivity in serum samples using LPS-coated microbeads (SFG or TG-specific) as a pre-treatment step, but the decrease in false-positive titers by MIF was less than a single two-fold dilution. Similar results were found using Weil-Felix antigens for adsorption.

**Abstract #40**

Reliability of the polymerase chain reaction for detecting *Rickettsia* spp. and *Rickettsia rickettsii* infection in patients from Sonora, Mexico.

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Rocky Mountain spotted fever (RMSF) is a public health problem with a reemerging pattern in several states across Mexico, in which is mainly transmitted by the tick *Rhipicephalus sanguineus* sensu lato. Delay in diagnosis and specific treatment may produce fatal outcomes. Confirmatory diagnosis is mainly based on the increase of immunoglobulin G (IgG) determined by indirect immunoassay (IFA), but several days are needed to show such increase. The polymerase chain reaction (PCR) may produce faster results than IFA and at the experimental level has shown a good diagnostic capacity, but there is no current evidence of its reliability at the population level. For such a reason we evaluated the reliability of PCR in blood samples of hospitalized patients from Sonora, Mexico. The diagnosis was made in samples of whole blood.
and serum, obtained from patients with clinical suspicion of RMSF. The sample was drawn in different days from onset of symptoms. The bacterium was identified by end-point PCR amplifying two genes: the gene $gltA$ for genus Rickettsia, obtaining a product of 401 base pairs, and the A1G_04230 for the species rickettsii, achieving a product of 153 base pairs. The results of 172 samples were compared with real-time PCR used by the Centers for Diseases Control and Prevention, a Kappa coefficient $\approx 0.78$ was observed. When samples ($n=443$) were compared with the National Reference Laboratory (InDRE), a Kappa coefficient $\approx 0.9$ was obtained. Using PCR, positive results were found in samples of whole blood and serum, drawn from days 1 to 15 from onset of symptoms. Negative results can be obtained from patients with $\geq 1$ day of treatment with doxycycline.

### Abstract #41
Identification and characterization of a novel adhering receptor for spotted fever group rickettsiae
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Spotted fever group (SFG) rickettsioses are tick-borne zoonotic diseases of global importance caused by obligatory intracellular bacteria of the genus Rickettsia. Although rickettsial infection is controlled by appropriate broad-spectrum antibiotic therapy, untreated or misdiagnosed SFG rickettsioses are frequently associated with severe morbidity and mortality. Endothelial cells (ECs) are the main mammalian host target cells of SFG rickettsiae. The most prominent pathophysiological effect is increased microvascular permeability, causing vasogenic cerebral edema and non-cardiogenic pulmonary edema with potentially fatal outcomes. The underlying mechanism(s) of rickettsial attachment to and anchoring on the endothelial cell luminal surface remains incompletely determined, including how the bacteria overcome shear stress from blood flow prior to host cell invasion. Here we examined the role of endothelial surface annexin A2 (ANXA2) during rickettsial adherence to human endothelial cells. We demonstrated that endothelial surface ANXA2 contributed to rickettsial attachment to endothelial cells as an adhering receptor. In vivo data from an anatomy-based in vivo quantitative bacterial adhesion analysis system revealed that global depletion of ANXA2 diminished rickettsial adherence to the blood vessel luminal surface. Investigation of a protein-protein interaction at a single-molecule level by atomic force microscopy (AFM) biomechanically characterized endothelial apical surface ANXA2 as a receptor for rickettsial adhesin out membrane protein-B to bind. Coupled with site-directed mutagenesis investigation of protein-living cell interaction with AFM probed phosphorylation of the N-terminus of ANXA as a switch to control rickettsial adhering. Our study, targeting the pivotal initial step in successfully establishing bacterial infection, delineates both biomechanical and biochemical mechanisms underlying rickettsiae hijack endothelial surface ANXA2 for their adherence to non-phagocytic vascular endothelial cells. E. chaffeensis exploits tandem repeat proteins to interact with host proteins to promote intracellular survival. $^*$Correspondence to: Bin Gong, MD, PhD; University of Texas Medical Branch, 301 University Blvd, Galveston TX 77555. Tel. 409-266-6682; Fax. 409-266-6810; Email: bigong@utmb.edu.

### Abstract #42
Development of a Rickettsia 364D-specific TaqMan assay
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Rickettsia 364D, also known as “Rickettsia philipii”, represents a unique rickettsial serotype that, although very similar genetically to prototypical strains of Rickettsia rickettsii, causes an illness clinically distinct from Rocky Mountain spotted fever (RMSF). The disease caused by Rickettsia 364D is known as Pacific Coast tick fever (PCTF) and is generally considered less severe than classical RMSF. There have been no reported human deaths due to PCTF although approximately 25% of cases required hospitalization. PCTF patients commonly exhibit an eschar at the tick bite site and rash is rare, while rash
is commonly found on RMSF patients and eschars are rare. To date Rickettsia 364D has only been detected in the Pacific Coast tick, Dermacentor occidentalis, and the bite of this tick has been implicated in several cases of PCTF. Although the overall prevalence of Rickettsia 364D is thought to be relatively low (~1%), isolated foci have been detected with prevalence rates as high as 13%. Due to the proven pathogenicity of Rickettsia 364D in humans and the unknown impact of D. occidentalis on public health, additional studies on the epidemiology and ecology of PCTF are warranted. To better address these issues, we have developed a sensitive and specific TaqMan assay for the detection of Rickettsia 364D. This assay targets a 62 bp region of the transcriptional elongation factor gene (nusG) and takes advantage of a 7-bp deletion in nusG of Rickettsia 364D that does not occur in the homologous segment in R. rickettsii. The specificity of the assay was tested against 11 isolates of Rickettsia 364D, 26 isolates of R. rickettsii, 26 additional species of Rickettsia, 10 near neighbors (Anaplasma, Ehrlichia, Orientia, and Bartonella), 9 tick species, 4 cell lines, E. coli, and human DNA. Additionally, this assay was shown to detect Rickettsia 364D in DNA of D. occidentalis ticks and clinical samples from patients with PCTF who were previously shown to be positive using other non-specific methods. This assay represents a novel tool for the detection of Rickettsia 364D in human and arthropods and could aide in study of the epidemiology and ecology of PCTF.

Abstract #43

Employing FDA-approved compounds to develop host-targeted anti-Rickettsia therapy

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Pathogenic Rickettsia parasitize the mammalian vasculature, and infection frequently results in mortality. To date there is only one line of defense against Rickettsia infections; antibiotics. Analysis of rickettsial genomes shows evidence of past events that resulted in resistance to the great majority of available antibiotics. As such, we have a tenuous grasp on controlling these pathogens, and the current treatment has significant potential for future failure. It is therefore imperative that we develop alternative treatment strategies to prevent or lessen the burden of Rickettsia infections before the real possibility of antibiotic resistant pathogens arises. As Rickettsia are obligate intracellular pathogens, the bacteria completely rely on host cells for growth. This fact highlights a potential underappreciated weakness of this type of bacteria. Namely, that modulation of specific host cell activities can make the intracellular environment inhospitable for bacterial growth. We hypothesize that chemical manipulation of host functions that contribute to rickettsial growth will drastically reduce bacterial pathogenesis. We refer to this strategy as “host-targeted” therapeutics, as we propose to modulate the host to inhibit bacterial proliferation. A major benefit of this approach is that all chemicals have previously been approved by the United States Food and Drug Administration (FDA), so the cost and time to use in human population is drastically lower than in situ drugs development. We have previously performed a screen of 640 FDA-approved compounds for inhibition of R. conorii growth in vitro. This initial screen identified a series of non-antibiotic chemicals that drastically reduce rickettsial burden in vitro, suggesting that targeting host pathways can affect bacterial fitness. A subset of these chemicals was further analyzed to determine 1) in vitro inhibitory concentration, 2) time course of effectiveness, 3) host species efficacy, and 4) breadth of Rickettsia species efficacy. These promising compounds will be applied to small animal models of Rickettsia infection to assess in vivo efficacy in preventing and treating infection. Together, our data supports a new approach to treating intracellular infections; namely host-targeted therapy to inhibit host functions that promote bacterial growth.

Abstract #44

Serological Assessment of Rickettsia rickettsii, R. typhi, and R. parkeri Species Reactivity in Patients with Suspected Rocky Mountain Spotted Fever

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Rickettsioses caused by Rickettsia species can lead to severe illness and death in the absence of timely and appropriate antimicrobial therapy. The presentation of nonspecific, early symptoms of rickettsial diseases make clinical diagnoses challenging even in endemic areas. Immunofluorescence antibody assay (IFA) has long been established as the reference standard for rickettsial disease confirmation; however, species-specific distinctions in serological testing remains
challenging due to antigen specificity. In the present study, we evaluate the reactivity of Rickettsia rickettsii, R. parkeri, and R. typhi antigens in patient samples submitted to the CDC Rickettsial Diagnostic Lab for Rocky Mountain Spotted Fever (RMSF) serologic testing. Samples from 95 individuals were tested for rickettsioses by IgG IFA. The sample set includes paired acute and convalescent samples from 84 individuals, and 11 singular samples with positive titers for R. rickettsii. Significant titers were determined at a screening dilution of 1:64. Of this group, 6.2% had antibodies against R. rickettsii, 66.5% for R. parkeri, and 23.5% for R. typhi. There were 4 patients with significant titers for all three species, and 30 patients with significant titers for at least two. A total of 23% of our testing results show possible cross-reactivity. Additionally, we have identified 108 samples for R. parkeri and 38 samples for R. typhi that were positive after testing negative for Spotted Fever Group (SFG) reactivity against R. rickettsii. This preliminary work will help to describe the specificity of these tests, and challenges the current diagnostic testing algorithm for assessing SFG rickettsioses. Further studies may improve understanding of the specificity of Rickettsia serology assays, and better define the etiology of SFG Rickettsioses.

Abstract #45
National Capacity for Rickettsia Molecular Detection
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The Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Rickettsial Zoonoses Branch (RZB), Rickettsial Diagnostics Team provides CLIA-compliant diagnostic testing of human clinical specimens and case related environmental samples, to support public health laboratories with difficult diagnoses, cases of illness with unknown etiologies, and laboratory outbreak response. We partnered with the CDC’s Division of Preparedness and Emerging Infections, Laboratory Preparedness and Response Branch (LPRB) to develop, validate, and deploy an FDA (510k) cleared assay kit to Laboratory Response Network –Biological (LRN-B) member laboratories which include approximately 130 US public health laboratories and foreign and domestic Department of Defense labs. Highly specific Rickettsia species, R. prowazekii, and R. rickettsii real-time PCR assays and a positive plasmid control were developed and validated through rigorous analytical and clinical testing of panels and samples. Three lots of diagnostic kits including positive controls were manufactured, quality control tested, and assessed for performance well as reproducibility. LPRB submitted the 510(k) to the FDA, and the Rickettsia assay received 510(k) clearance in October 2017. Proficiency test panels and kits were deployed to the requesting LRN-B laboratories by LPRB and CDC’s Division of Scientific Resources. Equipping LRN-B laboratories with this assay is critical to providing prompt and accurate diagnostic results. Understanding where tickborne diseases occur is critical in saving lives and directing public health resources to appropriate surveillance, prevention, and education efforts.

Abstract #46
Development of an Orientia genus-specific quantitative real-time PCR assay and the Detection of Orientia species in DNA preparations from O. tsutsugamushi, Candidatus Orientia chuto, and Orientia species from Chile
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Scrub typhus presence outside the Tsutsugamushi Triangle has been described in the Middle East, South America, and Africa. An Orientia agent isolated from a patient infected in Dubai, United Arab Emirates has been grown in culture and partially characterized; it is genetically distinct from O. tsutsugamushi and therefore believe to be a new species: Candidatus Orientia chuto. The presence of Orientia species from clinical isolates from Chile have been genetically characterized and several have been found to be unique when compared to O. tsutsugamushi and Ca. O. chuto. These three groups of Orientia species, O. tsutsugamushi, Ca. O. chuto and the molecular isolates from Chile are not detectable with a single assay currently available. We designed a quantitative real-time PCR (qPCR) assay, Orient16S, to detect agents from these three Orientia genogroups based upon their 16S rRNA gene (rrs) sequence. The assay was optimized, the
specificity of this new assay was tested with DNA preparations from O. tsutsugamushi (n=20), Ca. O. chuto (n=1), Chile isolates (n=3), Rickettsia species (n=18), and other bacteria (n=15). Orien165 targeting a 94bp fragment of rrs was capable of detecting DNA preparations from all three Orientia genogroups, but not DNA from near or far neighbor bacteria. This assay was sensitive enough to be able to detect Orientia DNA extracted from a serum sample of Chilean patient. The Orien165 qPCR assay specifically and sensitively detects O. tsutsugamushi, Ca. O. Chuto and Chilean orientiae DNA. Thus, this assay could be used to screen for orientiae from clinical and environmental samples in and outside the Tsutsugamushi Triangle.

Abstract #47
Modeling Spatial Distributions of Ticks and Tick-borne Diseases
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Description and analysis of variations in the geographic distribution of disease vectors and vector-borne pathogens is crucial to safeguarding public health. Spatial distributions of ticks are largely determined by climate due to them being poikilothermic ectoparasites, which renders their modeling a relatively straightforward exercise. Models therefore purporting to describe the present-day and future distributions of ticks (and tick-borne pathogens) can be found abound in the literature. However, the practice of spatial distribution modeling, particularly those using machine learning algorithms such as the popular maximum entropy (MaxEnt) approach, exist today mostly as an “art form”, and only recently, the modeling community has developed rigorous, quantitative methods that epidemiologists may benefit from deliberation. We took into consideration much of the recent recommendations and evaluated the present-day and future spatial distributions of three of the most medically significant tick species in North America; Ixodes scapularis, Amblyomma americanum and Dermacentor variabilis. Our results differ from conventional understanding of distributions of these species, and as well as from the predictions of their distribution by previous models in North America. In general, our results indicate a broader presence of these ticks than currently understood, some of which was recently confirmed via field surveys. Further, while climate change will render northern latitudes more suitable for the establishment of these ticks, a loss of conventionally known territories for these species can also be expected in the near future.

Abstract #48
Coxiella genetics: How far have we come?
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There are several obstacles to genetic transformation of obligate intracellular bacteria including efficient DNA uptake, selection, scoring, expansion, and cloning. C. burnetii is a natural obligate intracellular bacterium. However, we recently recused the pathogen from its eukaryotic host by development of acidified citrate cysteine medium (ACCM-1 and -2), which supports host cell-free (axenic) growth. This advance dramatically aided development of a robust genetics toolbox for C. burnetii that includes Himar1 transposons, shuttle vectors, gene reporter assays, counter-selectable markers, inducible gene expression, loop in/loop out and Cre-lox gene inactivation systems, and a miniTn7 transposon for site-specific transgene expression. A 3rd generation medium termed ACCM-D is nutritionally defined, which allowed development of nutritional selection systems based on arginine or lysine auxotrophy. This is a key advance considering the limited number of antibiotic resistance markers approved for use with select agent C. burnetii. These genetic tools have kickstarted C. burnetii research with several laboratories generating Himar1-based transposon libraries for virulence factor discovery. Our laboratory has generated over 50 C. burnetii deletion mutants in both virulent and avirulent strains which have defined gene products responsible for host cell modifications, lipid and peptidoglycan remodeling, and lipopolysaccharide phase variation. Advances of host cell-free growth and genetic manipulation of C. burnetii have resulted in a paradigm shift in how the organism is viewed and studied, i.e., a tractable facultative intracellular bacterium amenable to genetic manipulation. New genetic approaches are currently providing novel insight into C. burnetii macrophage parasitism and disease pathogenesis.
Host cell-free (axenic) culture of *Coxiella burnetii* in acidified citrate cysteine medium-2 (ACCM-2) has provided important opportunities for investigating the biology of this naturally obligate intracellular pathogen and enabled the development of tools for genetic manipulation. However, ACCM-2 has complex nutrient sources that preclude study of individual components required for *C. burnetii* growth. We developed a nutritionally defined medium termed ACCM-D to refine our understanding of *C. burnetii* growth requirements. Compared to ACCM-2, ACCM-D supported a longer logarithmic growth phase, a more gradual transition to stationary phase, and greater overall replication. Small-cell-variant morphological forms generated in ACCM-D also showed increased viability relative to those generated in ACCM-2. Amino acid-deficient formulations of ACCM-D revealed *C. burnetii* is auxotrophic for 11 amino acids, including arginine and lysine. Heterologous expression of *Legionella pneumophila* argGH and lysAC in *C. burnetii* permitted growth in ACCM-D missing arginine or lysine, thereby providing a nonantibiotic means of selection for *C. burnetii* genetic transformants. Consistent with bioinformatic predictions, the elimination of glucose did not impair *C. burnetii* replication. Using a comparative approach, we characterized differences in *C. burnetii* SCV peptidoglycan composition and found distinct differences in composition and cross-linking between SCV propagated intracellularly versus axenically (ACCM-2 or ACCM-D). Together, these results highlight the advantages of a nutritionally defined medium for investigations of *C. burnetii* metabolism, development, and virulence. Understanding how nutritional context influences *C. burnetii* biology will provide important insight into the host-pathogen dynamic.

**Abstract #50**

**Growth and analysis of *Coxiella burnetii* using axenic media**

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*Coxiella burnetii*, the bacterium that causes Q fever, replicates intracellularly in a paristophorous vacuole (PV) at low pH (<5). These replication requirements have limited study of *C. burnetii* and Q fever. In 2009, Omsland, et al reported growth of *C. burnetii* in a host cell-free culture medium (Acidified Citrate Cysteine Medium). This medium, ACCM, and its later iterations ACCM-2 and ACCM-D, has allowed significant advances in the ability to study *C. burnetii*, but limitations on the use of ACCM must be considered. *C. burnetii* isolates representing a broad spectrum of genotypes were grown in ACCM-2 and growth of some genotypes was found to be limited. The ST-8 genotype, which is commonly found in North American goats and Q fever patients, has very little replication in ACCM-2. Serial passage of Nine Mile Phase 1 *C. burnetii* in ACCM resulted in loss of complex LPS side chains and reduced virulence in a mouse model. Use of ACCM-2 agar plates is an effective method for enumerating viable *C. burnetii* and has been used to demonstrate rapid inactivation of *C. burnetii* at 70°C and after short exposures to disinfectants. An *in vitro* system to evaluate the efficacy of antibiotics on *C. burnetii* growth and survival showed that *C. burnetii* growth is highly sensitive to doxycycline in an axenic assay system, but doxycycline does not kill *C. burnetii*. Increasing the pH of ACCM-2 can be just as effective as doxycycline at reducing *C. burnetii* growth. ACCM and its derivatives have greatly facilitated *C. burnetii* research in recent years. Although there are some limitations with strain usage and maintenance of virulence, evaluation of the phenotypes of *C. burnetii* strains and genetic mutants is rapidly progressing.

**Abstract #51**

**Applying evolutionary insights to control *Coxiella burnetii* growth**

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The unique biphasic life cycle of *Coxiella burnetii*, the etiologic agent of acute Q fever and chronic endocarditis, includes a metabolically active intracellular form that occupies a lysosome-derived acidic vacuole. *Coxiella* is the only bacterium known to thrive in such an intracellular compartment, and this ability is fundamental to its pathogenicity; however, very
little is known about metabolites that are vital to \( C.\ burnetii\)'s intracellular growth. This lack of knowledge has hindered both the understanding of its intracellular physiology and the development of better therapies. In order to identify metabolic processes that contribute to \( C.\ burnetii\)'s intracellular growth and virulence, we interrogated its genome using evolutionary genomics approaches. Our analyses identified numerous metabolic genes that were acquired by \( C.\ burnetii\) from the environment, including genes required for the biosynthesis of heme and biotin. By generating mutant strains and by using molecules that block heme and biotin biosynthesis pathways, we show that the production of heme and biotin are critical to the growth of \( C.\ burnetii\). Collectively, our study demonstrates that foreign-origin genes boost \( C.\ burnetii\)'s physiology, and because several of these genes are important to \( C.\ burnetii\)'s biology but are not present in humans, they are ideal candidates for the development of new anti-\( C.\ burnetii\) therapeutics with potentially low side effects.

**Abstract #52**

**Development and effective utilization of axenic culture tools in the study of bacterial obligate intracellular parasites**

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Research on bacterial obligate intracellular parasites (BOIPs) is generally hampered by sub-optimal methods for host cell-free (axenic) culture and/or limiting tools for genetic manipulation. Strategies used in the development of platforms for axenic culture and genetic manipulation of \( C.\ burnetii\), the agent of Q fever, serve as a guide for development of similar research tools for use with other BOIPs. \( C.\ burnetii\) exhibits multifactorial physicochemical and nutritional requirements for growth and other BOIPs may show similar requirements. Indeed, absolute requirements for acidic pH, presence of CO\(_2\) and reduced O\(_2\) availability for optimal replication could explain the basis for niche restriction in \( C.\ burnetii\). Multifactorial growth requirements combined with relatively reduced metabolic plasticity likely increases the relative number of genes essential for replication with implications for mutant recovery. Consequently, use of nutritionally limited media increases the risk of loss of mutants with metabolic defects. For example, a \( C.\ burnetii\) mutant with an insertion in the gene \( pckA\), encoding phosphoenolpyruvate carboxykinase, the first committed step in gluconeogenesis, required chemical complementation with glucose for recovery. For \( C.\ burnetii\) and any pathogen with slow generation times, culture time causes significant research delays. Culture platforms that enhance replication and colony development reduce overall time for mutant generation and isolation. Pathogen-specific biology, including distinct infectious and replicative cell forms suggest an understanding of mechanisms for developmental transitions is necessary before axenic culture tools can be effectively employed with some BOIPs. For \( Chlamydia\ trachomatis\), transition between infectious and replicative cell forms has been postulated to be regulated via several mechanisms, including nutrient availability. The transcriptional regulator DksA is associated with the bacterial response to nutritional stress and serves a potential role in regulation of chlamydial physiology in response to nutrient availability.

**Abstract #53**

**Targeted mutagenesis broadly applicable in \( Ehrlichia\) species in creating mutations to disrupt and restore a gene activity, and also to introduce expression tags in proteins**

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Lack of an efficient system for targeted mutagenesis remained a major challenge in studying rickettsial pathogens, including members of the genera \( Ehrlichia\), \( Anaplasma\), \( Neorickettsia\), \( Orientia\) and \( Rickettsia\). In our recent study using \( Ehrlichia\ chaffeensis\), we developed protocols to generate stable targeted mutations by allelic exchange, and employed these techniques to inactivate two genes and restore one gene function (Wang et al., Sci. Rep. 2017). The mutagenesis methods are now being used in creating additional mutations at several gene targets within the \( E.\ chaffeensis\) genome, and in other related pathogens, such as \( Ehrlichia\ canis\) and \( Anaplasma\ phagocytophilum\). We have created additional mutations useful in monitoring inactivation of genes, protein expression, protein-protein interactions and protein-DNA interactions. We also created mutations resulting in attenuated bacterial strains for use as possible candidates for vaccine studies. The benefits of these improved methods, which are broadly applicable for genetic studies in rickettsials, will be discussed.
Abstract #54
Impact of Three Different Mutations in *Ehrlichia chaffeensis* in Altering the Global Gene Expression Patterns
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The rickettsial pathogen *Ehrlichia chaffeensis* causes a tick-borne disease, human monocytic ehrlichiosis. Mutations within certain genomic locations of the pathogen aid in understanding the pathogenesis and in developing attenuated vaccines. Our previous studies demonstrated that mutations in different genomic sites in *E. chaffeensis* caused variable impacts on their growth and attenuation in vertebrate and tick hosts. Here, we assessed the effect of three mutations on transcriptional changes using RNA deep-sequencing technology. RNA sequencing aided in detecting 66-80% of the transcripts of wildtype and mutant *E. chaffeensis*. Mutation in an antiporter gene (ECH_0379) causing attenuated growth in vertebrate hosts resulted in the down regulation of many transcribed genes. Similarly, a mutation downstream to the ECH_0490 coding sequence resulted in minimal impact on the pathogen’s *in vivo* growth, but caused major changes in its transcriptome. This mutation caused enhanced expression of several host stress response genes. Even though the ECH_0660 gene mutation caused the pathogen’s rapid clearance in vertebrate hosts and aids in generating a protective response, there was minimal impact on the transcriptome. The transcriptomic data offer novel insights about the impact of mutations on global gene expression and how they may contribute to the pathogen’s resistance and/or clearance from the host.

Abstract #55
The *Anaplasma marginale* msp2 pseudogene repertoire: multistrain genomic analysis provides insights to evolution and fitness
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*Anaplasma marginale* is an obligate intracellular rickettsia that establishes lifelong infection in ruminants. The pathogen achieves persistence by varying surface proteins to evade host immune responses. Major surface protein 2 (Msp2) has a central hypervariable region (HVR) that is surface exposed, flanked by conserved regions. Msp2 is encoded by a single operon linked, full length gene, however there are multiple (5-8) truncated pseudogenes throughout the genome that contain short flanking conserved regions and the HVR. Msp2 variation is effected by recombination of whole or partial pseudogenes into the expression site (ES) by a specialized recombination mechanism known as gene conversion. Gene conversion is a unidirectional “copy and paste” mechanism, whereby the pseudogenes remain unchanged and only the ES copy of the gene is changed. Once recombined into the ES the pseudogenes are expressed as functional protein. Therefore, these pseudogene repertoires arise under strong divergent selective pressures – one that selects for a diverse array of pseudogene alleles to provide maximum opportunities for immune escape and a second that constrains allelic diversity such that they retain function. Multistrain analysis of *A. marginale* msp2 repertoires reveals that there are eight conserved loci that harbor msp2 pseudogenes. Because these sequences are repeats, they do not come through with high fidelity in high throughput sequencing projects, therefore we devised a strategy to sequence the pseudogenes from several strains. Our results show that there are identical pseudogenes within (duplicated) and between (conserved) strains. Comparing strains reveals that some pseudogenes in the duplicated position have small changes, suggesting a scenario for evolution of the msp2 pseudogene repertoire: duplication of a pseudogene followed by the accumulation of sequence diversity. We hypothesize that the variant in the “duplicated” position would encode immune escape variants, resulting in a mechanism for expanding the repertoire of beneficial alleles, without loss of fitness.

Abstract #56
Global transcriptomics analysis of macrophages infected by a pathogen and a non-pathogen SFG *Rickettsia* reveals differential reprogramming of host signaling pathways
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Recent studies in several animal models have provided evidence of non-endothelial parasitism by different *Rickettsia* species suggesting that the interaction of rickettsiae with cells other than the endothelium may play an important role in the pathogenesis of rickettsial diseases. We have reported that two members of SFG *Rickettsia* have completely distinct intracellular fates in human THP-1 macrophages. Consequently, we expect early transcriptomic host alterations either as a response of host cells to tackle the infectious agent or as a result of pathogen modulation to make the intracellular environment more favorable for its lifestyle. We have herein performed a comprehensive transcriptomic profiling of THP-1 macrophages infected with a pathogenic (*R. conorii*) and a non-pathogenic (*R. montanensis*) SFG *Rickettsia*, at 1 hour post-infection. Our results revealed 470 and 86 differentially regulated host genes upon infection with *R. conorii* and *R. montanensis*, respectively; thereby anticipating major differences in macrophage responses to pathogen vs. non-pathogen *Rickettsia*. Transcriptional programmes generated upon infection with *R. conorii* point towards a sophisticated ability of these bacteria to modulate the expression of inflammatory signals, balancing the expression of host pro- and anti-inflammatory mediators and switching immune signals into a hyporesponsive state. Moreover, *R. conorii* was also able to induce the expression of several pro-survival genes, which results in the ability of *R. conorii* to prolong host cell survival, thus protecting its replicative niche. Our results also show a substantial manipulation of different regulators of the gene expression machinery, which may impact immediate and late responses during infection and may be key to *R. conorii* tropism for THP-1 macrophages. Our results provide new insights on the molecular mechanisms underlying the differential species-specific patterns of rickettsial cellular tropism and pathogenicity, opening several avenues of research in host-rickettsiae interactions.

**Abstract #57**

**Direct Sequencing of *Rickettsia* Plasmidomes from Ticks**

Arunachalam Ramaiah

Ticks harbor the second largest number of arthropod-borne pathogens transmitted to animals and humans. Of the well-studied arthropod pathogens, *Rickettsia* include more than 26 species and one or more plasmids are known to be present in nearly half of them. Genomes of only a few of the many tick bacterial agents that are not cultivable have been characterized by deep next generation sequencing (Illumina HiSeq). In order to fill the gaps in genome information and to better understand the evolution and potential role of plasmids in *Rickettsia*, we used the Ion Torrent Personal Genome Machine (IT-PGM) as an alternative approach for direct sequencing of *Rickettsia* genomes and plasmids in four tick species: *Amblyomma americanum*, *Amblyomma maculatum*, *Dermacentor variabilis*, and *Dermacentor occidentalis*. DNAs were extracted from single alcohol-preserved ticks; some DNAs were Repli-G amplified. Size fractionated DNA libraries were sequenced on single 318 or 316 IT-PGM chips with 200 bp chemistry. Results showed that three complete plasmid genomes were obtained for *R. amblyommatis* from each of two samples of *A. americanum*, and nearly complete single plasmids for *R. bellii*, *R. rhipicephali* and *R. andeanae* from *D. variabilis*, *D. occidentalis* and *A. maculatum*, but not in *R. parkeri* from *A. maculatum* which is not known to have a plasmid. Since the tick-derived *Rickettsia* plasmid sequences were highly homologous to sequences previously obtained from highly purified cell culture isolates of *R. amblyommatis*, *R. bellii*, and *R. rhipicephali*, these plasmids can be stably maintained after rickettsial isolation and propagation. The relatively inexpensive IT-PGM sequencing approach is a suitable method for rapid plasmidome characterization of *Rickettsia* species directly from ticks. Because the plasmidomes of *Rickettsia* have undergone substantial divergence, this new genetic information can support rapid development of new assays for differentiating species and for identifying new *Rickettsia* agents.
Abstract #58

Scrub typhus infection burden in Gorakhpur district, Uttar Pradesh, India: A community based study during lean and peak periods for Acute Encephalitis Syndrome

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In India, majority of Acute Encephalitis Syndrome (AES) outbreaks are reported from the Gorakhpur district in Uttar Pradesh, occurring predominantly during the monsoon season and affecting children. Annually, 1500 to 2000 AES patients get admitted to the BRD Medical College (BRDMC), Gorakhpur, the only tertiary care hospital in the region, with a case-fatality ratio of 20 to 25%. Scrub typhus, though identified as the predominant etiology for acute encephalitis syndrome (AES) outbreaks in Gorakhpur, the scenario at community level remains largely unexplored. In order to delineate the extent of background scrub typhus infection prevalence and associated risk factors, we conducted community-based serosurvey in two phases during lean and peak period of AES outbreaks and also estimated the incidence of infection. Total 2245 individuals were surveyed. Scrub typhus seroprevalence during peak AES period (IgG-70.7%, IgM-8.4%) was significantly higher as compared with lean period (IgG-50.6%, IgM-3.4%). The factors independently associated scrub typhus IgG positivity during lean period were female gender (AOR: 1.6 (CI 1.2, 2.3), p=0.004), not wearing foot wear (AOR: 1.9 (CI 1.1, 3.3), p=0.015), no bath after work (AOR:1.5 (CI: 1.03, 2.2), p=0.034). The risk factors during peak period were increasing age (Age 16-25 AOR: 2.5 (CI:1.6,3.9), P=0.000, Age>25 yr, AOR 2.5 (CI:1.3,5) p=0.005), female gender (AOR: 1.6 (CI:1,2,4), p=0.017), illiteracy (AOR:1.9 (CI:1,1,3,3), p=0.013), animal contact (AOR:1.6 (CI:1,1,2,3), p=0.004) and open air defecation (AOR:1.6 (CI:1,1,2,3)). None of the risk factors was independently associated with IgM scrub typhus positivity in any period. IgM positivity among fever cases was significantly more as compared with no fever respondents (11.8% vs 7.5%, p=0.023). Incidence of scrub typhus infection was 19.7% (CI:15,25%) and subclinical infection rate was (54%, 95% CI [39%, 68%]). Our study confirms the endemicity of scrub typhus in Gorakhpur, reports risk factors and proposes its control measures.

Abstract #59

Community-based prevention of Rocky Mountain spotted fever, Sonora, Mexico, 2016

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Rocky Mountain spotted fever (RMSF) transmitted by brown dog ticks (Rhipicephalus sanguineus sensu lato) is a public health problem in Sonora, Mexico. During 2009-2015 there were 56 RMSF cases and 22 deaths reported in a single locality (Community A). Sustained high case rates and deaths warrant interventions to limit exposure to the tick vector. An intervention was carried out in community A from March 2016 through November 2016 aimed at reducing tick populations with long-acting tick collars on dogs, environmental acaricides applied every month to peri-domestic areas, and RMSF awareness education. Tick levels were measured by inspection of community dogs every other month to monitor the efficacy of the project. A similar neighborhood (community B) was selected as a comparison control. By the end of the intervention the percentage of dogs with visible tick infestation was 8.8%, almost 4 times less than the initial proportion (32.5%) (p<0.01). In comparison, in the community B the percentage of dogs with visible tick infestation decreased from 19% at the start of the intervention to 13.4% at the end of the project, but this was not statistically significant (p=0.36). During the study period, no RMSF cases were reported from the intervention area; meanwhile two RMSF confirmed cases were reported from community B, including one fatal case. Community based interventions using an integrated approach and community education can prevent human illness and deaths from RMSF transmitted by Rhipicephalus sanguineus.

Abstract #60

Glucose-6-phosphate dehydrogenase deficiency and fatal Rocky Mountain spotted fever in Brazil and the U.S.

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In Brazil and the U.S., fatal Rocky Mountain spotted fever (RMSF) is associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency. We report fatal RMSF in the U.S. and Brazil in G6PD-deficient patients and suggest mechanisms for fatal RMSF in G6PD-deficient patients.
Glucose-6-phosphate dehydrogenase (G6PD) is a crucial enzyme in the metabolic pathway that produces the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), a biomolecule that protects all cells against oxidant agents. At least 186 distinct mutations have been identified in the G6PD gene that encode for enzyme variants with a wide spectrum of diminished, relatively normal, or sometimes increased activity. G6PD deficiency has been associated with increased severity of several rickettsial diseases including scrub typhus, murine typhus, Mediterranean spotted fever, and Rocky Mountain spotted fever (RMSF). Fulminant RMSF, an unusually severe and accelerated presentation of RMSF that progresses to death within 3-5 days of illness onset, has been linked to G6PD deficiency. Within the United States, estimated contemporary case fatality rates of RMSF range from ~5% to 10%, whereas those in Brazil exceed 40% in some states. The reasons for apparent differences in disease severity are incompletely understood. To explore the role of G6PD deficiency as a possible factor influencing fatal RMSF in Brazil, we evaluated formalin-fixed, paraffin-embedded tissues from 75 Brazilian and 69 US patients with fatal RMSF for two frequently identified point mutations in the G6PD gene, using a commercially available Taqman assay to detect substitutions at loci 202 or 376. Mutations were detected at 202, 376, or both, in 3 (4.3%), 6 (8.4%) and 3 (4.5%), respectively, of patients for whom PCR was successful. By comparison, only 1 (1.5%) US patient had a mutation at either locus. Median survival times of persons with a mutation at either locus were significantly shorter than those with the wild-type genotype. Additionally, significant differences were identified between the Brazilian and US cohorts in the percentage of male patients, median age at death, and percentage of fulminant infections. Various host factors, including the frequency of G6PD deficiency in the general population, likely contribute to differences in RMSF case fatality rates observed between Brazil and the United States.

**Abstract #61**

**Evaluation of Multiple Chlortetracycline-Medicated Mineral Formulations to Control Active Bovine Anaplasmosis in Endemic Cattle Herds.**

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Bovine anaplasmosis the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production. In the United States (U.S.), bovine anaplasmosis is conservatively estimated to cost the cattle industry >$300 million per year. The only approved antimicrobial treatment for bovine anaplasmosis in the U.S. are tetracycline antimicrobials. These antimicrobials have been demonstrated effective in controlling acute anaplasmosis but not chemosterilization, at recommended therapeutic doses. There are currently no antimicrobials labeled for the elimination of persistent *A. marginale* infection in carrier animals. Alternative strategies to control anaplasmosis include: maintenance of *Anaplasma marginale*-free herds, immunization against *A. marginale*, and intensive vector control; however, these strategies are largely impractical or not efficacious. In the absence of approved vaccines and ineffective *A. marginale* control alternatives, anaplasmosis control in endemic areas is predicated on administration of low doses of chlortetracycline (CTC), usually supplied in feed or mineral supplements for several months or longer. There are currently four U.S. Federal Drug Administration (FDA)-approved free-choice CTC-medicated mineral formulations (700, 5,000, 6,000, 8,000 g/ton). In a Kansas statewide survey, 59% (460/782) of producers indicated that they administer CTC-medicated feed for an average of 6 months per year to control active anaplasmosis. To determine whether one of the free-choice CTC-medicated mineral formulations provides superior protection against anaplasmosis, groups of cattle living in *A. marginale* endemic areas were treated with different CTC-medicated mineral formulations for six months. Anaplasmosis status was monitored monthly by PCR, competitive ELISA, and clinical disease evaluation. Blood plasma chlortetracycline levels were also monitored monthly. As tetracycline antimicrobials are the only FDA-approved antimicrobials to treat *A. marginale*, studies critically assessing the efficacy of this medically-important antimicrobial to control bovine anaplasmosis are needed to inform science-based policy recommendations, and overall, to improve antimicrobial stewardship, mitigate development of resistance, and protect human health.
29th Meeting of the American Society for Rickettsiology

Monday, June 18, 2018

Abstracts #62-118

Outside of the box - CRISPR, host-associated bacteria, Chlamydia

Poster Session B: Genomics, Transcriptomics, Proteomics, Host, Vector, Pathogen Interactions, Pathogenesis/Pathophysiology/Cell Biology, Immunity/Vaccine Development

Immunity and Vaccine Development

Pathogenesis, Pathophysiology, and Cell Biology I.

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**Abstract #62**  
**Genetic Manipulation Strategies for Chlamydia: Status, Strengths, and Limitations**  
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*Chlamydia* has finally joined many bacterial fields by acquiring most of the conventional tools for genetic manipulation. Two of the key advances, a transformation method and a shuttle vector with selectable markers occurred about 7 years ago, enabled the development of additional forward and multiple new reverse genetic manipulation strategies. These tools are now being utilized by scientists to investigate the genetic correlates for numerous biologic and pathogenic aspects. This review will briefly highlight the history of these developments along with the specific tools that have been developed. It will also highlight recent accomplishments using transposon mutagenesis and lateral gene transfer for the discovery of novel virulence factors. Lastly, limitations and factors to consider as the field continues to incorporate these tools and strategies will be discussed.

**Abstract #63**  
**Expanding the bacterial genetics toolkit with CRISPR interference**  
Jeremy Rock  
Laboratory of Host-Pathogen Biology, Rockefeller University, New York, NY 10065

*Mycobacterium tuberculosis* (Mtb) is the leading cause of death due to infectious disease and infects approximately one-quarter of the world. To facilitate genetic approaches in Mtb, we recently developed an improved CRISPR interference (CRISPRi) system for mycobacteria. CRISPR interference (CRISPRi) repurposes the CRISPR-Cas9 immune system for the sequence-specific control of gene expression. Here, I will discuss our approach to develop robust CRISPRi in Mtb (what worked and what didn’t) and provide evidence that this approach is generalizable. I will further discuss applications of CRISPRi in Mtb that leverage unique features of this platform, including the ability to silence multiple genes simultaneously as well as generate allelic series of knockdown mutants. Finally, I will provide an overview of potential extensions of this system to expand our genetic toolkit for intracellular bacteria.

**Abstract #64**  
**Advancing CRISPR-based tools in non-model bacteria**  
Chase Beisel  
Helmholtz Institute for RNA-based Infection Research, RNA Synthetic Biology Group, Würzburg, Germany

CRISPR-Cas systems and their RNA-directed nucleases have offered revolutionary genome editing technologies. The vast majority of the advances with CRISPR have occurred in eukaryotes, where CRISPR-based tools are now available for efficiently editing genome sequences, controlling gene expression, and performing combinatorial, genome-wide screens. Unfortunately, advances in bacteria have lagged—particularly in non-model bacteria where CRISPR still often remain to be implemented. The reasons for this lag are diverse but often boil down to species-specific and even strain-specific factors that are less problematic in eukaryotes, such as the efficiency of DNA transformation and homologous recombination, the active modes of DNA repair, and the cytotoxicity of the CRISPR nuclease. Developing a better understanding of these factors and how to overcome them has the potential to bring CRISPR technologies to a much larger swath of the microbial world and bring about the scientific advances already being realized in eukaryotes. As part of this talk, I will provide my perspective on the opportunities and challenges of implementing CRISPR nucleases in non-model bacteria. I will provide anecdotal examples from my own research group while providing suggestions and insights for developing genetic tools in Rickettsia. My goal is to provide a rough roadmap of how to develop CRISPR-based tools in this intracellular pathogen and what could be achieved once these tools are in hand.
Abstract #65
Whole Genome of “Rickettsia parkeri” strain Atlantic Rainforest-Like” Isolated from a Colombian Tick

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*Rickettsia* is an alphaproteobacterial genus transmitted by arthropods. This genus is divided into four groups: the ancestral group (AG), typhus group (TG), transitional group (TRG) and spotted fever group (SFG). *Rickettsia parkeri*, which is classified as a member of the SFG, has been reported across the American continents as infecting different tick species including the *Amblyomma maculatum* species complex, *A. ovale*, *A. nodosum* and *A. parvitarsum*. Different *R. parkeri* strains have been described in South America, including the Atlantic Rainforest strain isolated in Brazil. We have isolated a strain similar to this Brazilian strain from an *A. ovale* tick collected in Colombia. Our aim is to identify genetic and pathogenic characteristics of the new *Rickettsia* strain called “*Rickettsia parkeri*” Atlantic Rainforest-Like” (RpARFL). Vero-grown low-passage RpARFL was purified by renografin gradient centrifugation, and its DNA was used to sequence the whole genome by Illumina and MinION. Preliminary analysis has shown that RpARFL has several insertions, deletions and inversion zones in comparison to the *R. parkeri* Portsmouth type strain, and includes bacterial conjugation genes including TraC-, TraB-, TraU- and Tran-like genes. The sequence has 1,348,030 bp that is 47,644 bp longer than the *R. parkeri* Portsmouth strain genome. We are currently evaluating whether these genetic variants contribute to pathogenicity in a murine model. With this work, we expect to be able to distinguish differences among strains of the same species and how these changes affect pathogenicity in the animal model.

Abstract #66
Time-dependent, post-infection changes of serum metabolites from mice challenged with *Orientia tsutsugamushi*-infected mites

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*Scrub typhus* is an acute zoonosis caused by the obligate intracellular Gram negative bacterium, *Orientia tsutsugamushi* (OT). An in vitro chigger feeding mouse model was developed using ICR mice and *Leptotrombidium chiangraeiensis* (Lc1) chigger infected by OT. ICR mice were infected by feeding with OT-infected Lc1 (OLc1) chiggers. Serum was collected at 7 different time points from 6hrs to 10 days post initiation of feeding (PIF). Similarly, serum from control group of mice fed with naïve Lc1 chiggers (i.e. not infected by OT, NLc1) was also collected at 2 time points (6 hrs and 10 days) PIF. Metabolites in each serum were extracted and analyzed by UPLC-MS/MS. Biochemicals were identified and quantified using a library of chemical standards. Welch’s two sample t-tests (P-values ≤0.05) was used to identify those metabolites with significant variations in quantity at different time PIF and between the control group (NLc1) versus experimental groups (OLc1). The appearance of *Orientia* specific antibodies in these sera was monitored by ELISA. A number of biochemicals linked to immune function were found to be significantly altered between mice infected by the NLc1 and OLc1 chiggers, including itaconate, kynurenine, and histamine. Several metabolites, including glucose, pyruvate, lactate, that are linked to energy production were also found to be altered in the animals. Furthermore, time-dependent significant changes of these metabolites in OLc1 infected mice were only observed between day 7 and 10 PIF when the mice started to show signs of sickness. Global metabolomic characterization has revealed significant differences in the biochemical profiles of mice infected by the NLc1 versus OLc1 chiggers. The concurrent of significant changes in several metabolites, the initial appearance of sickness, and the detectable level of antibodies has suggested that a complicated and systematic biological interplay is contributing to the progression of disease in this feeding model.
**Abstract #67**

**RARP2 of Rickettsia rickettsii is transcribed early and at high levels**

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The Rickettsial Ankyrin Repeat Protein-2 of *R. rickettsii* is a type IV secreted effector that associates with ER membrane. RARP2 is truncated in the avirulent Iowa strain of *R. rickettsii* relative to that of the highly virulent Sheila Smith strain. The Iowa strain has an internal deletion of 7 of the 10 ankyrin repeat units that are found in the Sheila Smith strain. We used RNA-seq to demonstrate that RARP2 is highly expressed at timepoints as early as 4 hr post-infection and that these levels are maintained at 24 and 48 hr p.i. These results were confirmed by RT-qPCR which show that *rarP2* is transcribed at levels equivalent to *dnaK* from both the Sheila Smith and Iowa strains. These findings imply that RARP2 may play a role early in infection.

**Abstract #68**

**Rickettsial Shuttle Vectors With Expanded Host Range Reveal Mechanisms for Maintenance of the *R. monacensis* plasmid pRM.**

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Native plasmids of *Rickettsia amblyommatis* AaR/SC have previously been used to create a series of shuttle vectors for genetic manipulation of several *Rickettsia* species. However, attempts to transform the donor species were never successful. To improve our understanding of the mechanisms of rickettsial plasmid maintenance in diverse species, and increase the availability of genetic tools, we cloned the complete pRM plasmid containing a cassette encoding resistance to rifampin and a green fluorescent protein (Rif/GFPuv) from *Rickettsia monacensis* mutant clone 658. A series of shuttle vectors were created comprising various portions of pRM, and the selection cassette was replaced with one conferring resistance to spectinomycin (Spec/GFPuv). *R. amblyommatis* AaR/SC, *R. monacensis*, *Rickettsia bellii*, and *Rickettsia parkeri*, the *Ixodes pacificus* symbiont (REIP) and *Rickettsia montanensis* were successfully transformed with basic shuttle vectors containing pRM *parA* and *dnaA*. PCR assays targeting pRM regions not included in the shuttle vectors indicated that native pRM was still present in *R. monacensis* transformants at an approximately equal ratio to the shuttle vector. Determination of native pRM copy number indicated that only *R. monacensis* transformant pRM#4 contained a reduced number of the native plasmids with an average of 0.285 copies pRM per chromosome equivalent. Restriction analysis of plasmids recovered from shuttle vector-transformed *R. monacensis* revealed multiple instances of recombination between native and shuttle plasmids. In conclusion, we have created new shuttle vectors able to transform plasmid-free *Rickettsia* spp. as well as rickettsiae carrying native plasmids, including *R. amblyommatis* AaR/SC which was previously resistant to shuttle vector transformation. Recombination between native and shuttle plasmids in *R. monacensis* sheds light on the molecular mechanisms of plasmid maintenance in rickettsiae.

**Abstract #69**

**Survey of sheep herds’ Anaplasmataceae bacteria in Senegal**

 Mamadou Lamine Djiba, Senegalese Institute for Agricultural Research (ISRA)

Anaplasmataceae is one of three officially recognized families of the order Rickettsiales-α-Proteobacteria. (Dumler et al.2001). All representatives of these genera are obligate intracellular parasites of vertebrates and invertebrates, and some of them are etiological agents of arthropod-borne diseases of mammals. Anaplasma ovis, Anaplasma marginale, and Anaplasma moderately pathogenic in small ruminants. Ovine anaplasmosis has been observed in domestic and wild animals worldwide, including Europe (Ioannou et al.2011), China (Liu et al. 2012), and the USA (de la Fuente et al.2006 emerging tick (Ixodes spp.)-transmitted disease, human granulocytic anaplasmosis (Dumler et al.2001), as well as canine and equine anaplasmoses. It has been described in the USA, The objective of this study was to identify the causative agents of anaplasmosis and ehrlichiosis in sheep at two different sites in Senegal. The smears were then observed under light microscopy.Genomic DNA from blood was extracted using the QIAamp® DNA extraction kit (Qiagen, Hilden, Germany)
according to the manufacturer's instructions. We used the primers EHR16SR and EHR16SD to amplify a portion of the 16S rRNA gene of the most representatives of the Anaplasmataceae family (Parola et al. 2003). Using GenBank sequences of the groEL gene, we designed degenerate primers to amplify bacteria from the Anaplasma genus (ANGroElf 5'- GAG GCC ATC ACA GAT GG A5G-3' and ANgroElr 5'-CGG AAC TGC ATA TCA CCR TCA GT-3'). Amplification reactions were performed with a DNA thermal cycler as described elsewhere (Parola et al.2003). Materiel and Methods: The study was performed in two areas of Senegal: the Niayes and the Casamance (Ziguinchor). In each of the three sites, 150–200 sheep were included in the study for a total of 520 animals. All animals with the following clinical signs of acute infection were included in the study: hyperthermia, anemia, rapid weight loss, staggering, and paresis of the hind limbs. Blood samples from all ill animals were collected and examined prior to storage in liquid nitrogen for further molecular studies. The smears were then observed under light microscopy. Genomic DNA from blood was extracted using the QiAamp® DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We used the primers EHR16SR and EHR16SD to amplify a portion of the 16S rRNA gene of the most representatives of the Anaplasmataceae family (Parola and coll., 2003). Using GenBank sequences of the groEL gene, we designed degenerate primers to amplify bacteria from the Anaplasma genus. A total of 306 bp from the 16S rRNA which was used for construction of a phylogenetic tree. Results and discussions: Microscopic examination of only 52 of 120 blood smears of sheep revealed small, spherical intraerythrocytic inclusions that are suggestive of infection by Anaplasma or Ehrlichia spp. In 48/52 cases, we successfully obtained one or two amplicons from the blood samples. In ten cases, we were able to identify the species, because of the 100 % identity of the amplified gene which has been deposited in the GenBank. In six cases, it was Ehrlichia ruminantium, and in two cases, Anaplasma phagocytophilum. In two other cases, it was Anaplasma platys and a yet uncultured Anaplasma sp. Anaplasma phagocytophilum found in samples nos. 34 and 38 were identical to the most of the Anaplasma phagocytophilum originated from Europe and Asia conserve in the GenBank database.

Abstract #70
The effect of iron starvation of tick cells on A. marginale replication
Muna Solyman Ageli1, Debra C. Alperin1, Jessica Ujzco2, Susan M. Noh2
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Obligate intracellular, tick-borne, bacterial pathogens cause a variety of diseases in animals and humans. Ticks present a unique niche for pathogens for two reasons. First, unlike other hematophagous invertebrates, digestion of the blood meal occurs intracellularly rather than in the gut lumen. Second, ticks feed solely on blood, which is rich in iron due to the iron-containing hemoglobin. Iron is an essential nutrient for the growth and replication of most organisms and must be tightly regulated to prevent the formation of oxidative radicals, which damage cellular components. Little is known about iron uptake and regulation in ticks and how these processes affect tick borne, bacterial pathogens. Our long-term goal is to determine the affect of iron starvation in the tick on A. marginale replication. Anaplasma marginale, the causative of bovine anaplasmosis, is an obligate, intracellular gram-negative bacterium that infects erythrocytes and is transmitted by Ixodid ticks, including Dermacentor andersoni. In both the erythrocyte and tick midgut, A. marginale resides in an iron-rich environment due to the presence of hemoglobin. However, actual iron availability and its requirement for replication of A. marginale are unknown. We used an iron chelator 2,2'Bipyridyl (Bpd1) to starve DAE100 cells of iron. To measure successful starvation, we measured iron levels as well as transcript and protein levels of dmt1 and fer1, two tick genes central to iron regulation in the tick, in treated and untreated DAE100 cells. We will present the results of iron starvation in tick cells using Bpd1.

Abstract #71
Neutralization of Ehrlichial transmission from tick to mammalian cells by anti-Ehrlichial Invasin Protein, EtpE antibody
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Ehrlichia chaffeensis is an obligatory intracellular pathogen causing human monocytic ehrlichiosis, an emerging disease of human transmitted by the Lone star tick, Amblyomma americanum. Recently, our lab discovered novel functions of entry triggering protein of Ehrlichia (ECH_1038; EtpE) and its host receptor, DNaseX. This ligand-receptor complex facilitates
receptor-mediated Ehrlichial entry into mammalian host cells by inducing actin polymerization and blocking reactive oxygen species generation. Immunization of mice with recombinant EtpE and antibody against EtpE block ehrlichial infection of mammalian cells. In this study, we aimed to investigate whether antibody against EtpE is capable of inhibiting ehrlichial transmission from infected tick cells to human cells. The EtpE gene and protein were expressed by *E. chaffeensis* in ISE6 tick cells. Antiserum against C-terminus of EtpE (EtpE-C) significantly inhibited *E. chaffeensis* transmission from infected tick cells to mammalian cells compared to control serum. EtpE mRNA was highly expressed by *E. chaffeensis* in the adult Lone star ticks infected as nymph compared to both ISE6 and THP-1 cell lines. These data suggest antibodies against EtpE-C may be able to interfere *E. chaffeensis* transmission from infected ticks to mammalian hosts.

**Abstract #72**

*Orientia tsutsugamushi* Negatively Regulates Major Histocompatibility Complex I Expression via Reduction of the Transcription Factor NLRC5

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*Orientia tsutsugamushi* is an obligate intracellular pathogen and the causative agent of scrub typhus, a disease that threatens one billion and afflicts one million people annually. Control of *O. tsutsugamushi* involves major histocompatibility complex I (MHC-I) antigen presentation to activate cytotoxic CD8+ T cells (CTLs). Many intracellular pathogens, most notably viruses, employ a myriad of mechanisms to inhibit MHC-I expression and combat CTL-mediated surveillance, effectively camouflaging infected cells from the immune system. Here, we demonstrate that *O. tsutsugamushi* does so in a novel manner, targeting the master regulator of MHC-I expression, the transcription factor NLRC5. Infected HeLa cell lysates demonstrate lower levels of NLRC5 compared to uninfected cells, even during stimulation with IFNγ. This correlates with decreased transcript and protein levels of the MHC-I components, human leukocyte antigen A and Beta-2 microglobulin. Notably, *O. tsutsugamushi* infected cells exhibit elevated levels of *nlrc5* mRNA, suggesting that the bacterium post-transcriptionally targets NLRC5. To further define this mechanism, infected and control cells were treated with the eukaryotic translation inhibitor, cycloheximide, or the proteasome inhibitor, MG132. The ability of *O. tsutsugamushi* to reduce NLRC5 persisted with cycloheximide treatment, but was negated by MG132. Thus, *O. tsutsugamushi* promotes proteasomal degradation of NLRC5, reducing cellular levels of MHC-I. Because *O. tsutsugamushi* infects multiple cell types *in vivo*, with a range of antigen presentation capabilities, we repeated these experiments in monocytic THP-1 cells. The ability of *O. tsutsugamushi* to post-transcriptionally reduce NLRC5 and inhibit expression of MHC-I was recapitulated in THP-1 cells. However, the duration of inhibition was shorter, suggesting potential cell type-specific differences in this phenomenon. This is the first example of a microbe targeting NLRC5 to negatively regulate MHC-I expression, one that could be a shared immune evasion strategy among pathogens.

**Abstract #73**

Assessment of the Pathogenicity of Symbiotic Rickettsia in Guinea Pigs

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Many rickettsial species considered non-pathogenic/endosymbiotic are categorized as such based primarily on the absence of human or animal cases caused by them. However, there is mounting evidence that at minimum these bacteria can cause seroconversion in dogs and humans. This experiment aimed to assess the capability of three rickettsia- *Rickettsia amblyommatis*, *R. bellii* and *R. montanensis* to cause clinical signs, serological conversion, or lead to PCR detection in model animals. Hartley strain guinea pigs were separated into 4 groups of 3 animals each. Groups were exposed via IP inoculation to one of the three non-pathogenic rickettsia or *R. rickettsii*. Inoculum loads varied between $10^5$ for *R. bellii* Yolo to $10^7$ for *R. amblyommatis* Lake Alexander, *R. montanensis* OSU, and *R. rickettsii* AZ3. Animals were monitored for 13 days post-inoculation for clinical signs including scrotal edema and dermatitis. Additionally, whole blood and ear skin biopsies were taken every 2-3 days for PCR testing. At the time of euthanasia, organ samples were taken for additional PCR identification of rickettsia. This experiment was replicated for a second round with the same set-up, but without a *R. rickettsii* group. Serological conversion was seen in all groups, but with differences in the number of animals that
seroconverted and titers. Ear skin samples were PCR positive in all groups (1/6 *R. amblyommatidis*, 2/6 *R. bellii*, 3/3 *R. rickettsii*, and 3/6 *R. montanensis*). *R. montanensis* and *R. rickettsii* groups had rickettsial DNA in organ samples, with 2/6 and 3/3 animals having positive spleen samples, respectively. Clinical signs were noted in all groups, ranging from mild orchitis in *R. amblyommatidis* groups to significant orchitis and dermatitis in the *R. montanensis* and *R. rickettsii* group. These results would indicate that, at least in high doses, some rickettsia labelled as non-pathogenic/endosymbiotic can cause documentable infections in model animals.

**Abstract #74**

**Dynamics of rickettsial load in tick salivary glands and saliva during tick feeding**

Chanakan Suwanbongkot¹,³, Ingeborg Langohr², Wellesley Dittmar¹, Emma Harris¹, Rebecca Christofferson² and Kevin Macaluso⁴

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The discovery of pathogenic Rickettsia species, both novel pathogens and those that were previously considered to be rickettsial symbions has led to an increased reported rickettsial infections in the United States. Our laboratory studies demonstrate the transmissibility of rickettsial symbiont, *R. andeanae*, to vertebrate hosts during tick blood meal acquisition; however, the rickettsial loads associated with tick feeding remained unknown. Using *R. parkeri* or *R. andeanae*-infected *Amblyomma maculatum* cohorts, we hypothesized that known rickettsial pathogens will be transmitted in larger number when compared to rickettsial symbions. In this study, a qPCR assay was employed to quantify rickettsial load in tick salivary glands, saliva and vertebrate host at tick attachment site in different time points of bloodmeal acquisition. *R. parkeri* presence is significantly greater in tick salivary glands (~2-fold), saliva (~1.5-fold) and in the host skin at tick attachment site (~7.5-fold), compared to *R. andeanae* in over the course of tick feeding. Additionally, immunofluorescence assay using anti-RC<sub>PFA</sub> polyclonal antibody demonstrated the presence of both rickettsial species in tick salivary glands, however only *R. parkeri* were identified in vertebrate hosts by immunohistochemistry. Sustained rickettsial infection and subsequent disease mechanisms have not yet been elucidated, but the results of this study suggest successful transmission may be determined by total rickettsial load.

**Abstract #75**

**Nuclear Trafficking of *Orientia tsutsugamushi* effector Ank13**

Haley E. Adcox* and Jason A. Carlyon

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The causative agent of scrub typhus, *Orientia tsutsugamushi*, is a pathogenic obligate intracellular bacterium that poses an under-recognized global health threat. Intracellular survival of *O. tsutsugamushi* is linked to secretion of its remarkably large repertoire of ankyrin repeat-containing proteins (Anks) via Type 1 Secretion System machinery. When ectopically expressed, the Anks localize to distinct regions within host cells, and those characterized thus far have been shown to modulate diverse host cellular processes to the bacterium’s advantage. Ank13 uniquely demonstrates a nuclear localization phenotype. Given the precedent of nucleus-tropic effectors rewiring host cell gene expression profiles in a pro-microbial fashion, Ank13 warrants further study. While we previously reported that *O. tsutsugamushi* expresses ank13 during infection of host cells, how it trafficks to and enters the nucleus has not been determined. There are several reported pathways for nuclear translocation, the most well-defined of which relies on recognition of a nuclear localization signal (NLS) by importin. Ank13 contains a putative NLS in its N-terminal 49 residues similar to the consensus monopartite NLS. Removal of this region failed to alter nuclear translocation of Flag-Ank13 in HeLa cells, suggesting an alternative mechanism. The importin-independent RaDAR nuclear import pathway is based on the structural interaction of RanGDP with hydrophobic residues at the 13<sup>th</sup> positions of two consecutive ankyrin repeats (ARs) of a protein. Ank13 contains eight ankyrin repeats, with AR4 and AR5 each containing a hydrophobic residue at position 13. Experiments to investigate the significance of these residues to Ank13 nuclear translocation are currently underway. Identifying the mechanism of nuclear import will begin to elucidate Ank13’s function as an *Orientia* effector in host cell modulation.
Abstract #76
Culture of *Rickettsia felis* isolated from a tick
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2Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University

The isolation of various strains provides the possibility to study the biological properties, diversity and epidemiology of rickettsiae. Although *Ctenocephalides felis* has been revealed as the main vector of *Rickettsia felis*, additional flea, tick, mite and louse species have been associated with this bacterium in the literature. The ability of *R. felis* to infect multiple hosts and potential vectors can be explained by co-feeding transmission among different arthropods located on the same host. We succeeded in culture isolation of *R. felis* from a host-seeking *Ixodes ricinus*, the most frequent tick in Slovakia. The bacterial isolation was performed on XTC-2 cell line at 28°C using shell-vial technique. Evaluation of growth properties and propagation was made on both XTC-2 and Vero cell lines. Also, we observed *R. felis* microscopically by Gimenez staining in infected host cells and confirmed it serologically by immunofluorescence assay. New *R. felis* isolate was purified by gradient ultracentrifugation and visualized by scanning electron microscopy. Fragments of the genes gltA, ompA, ompB, htrA, rpoB, sca4, rffE and rrs were sequenced and compared with the corresponding sequences of the type strain URRWXCaI2 and other reported culture isolates. We did not spot any nucleotide polymorphism, however plasmid pRFδ, characteristic for the standard strain, was absent in our isolate. Herein, we describe the first successful isolation and characterization of a tick-derived *R. felis* strain “Danube”, obtained from an *I. ricinus* nymph. Acknowledgement: This study was financially supported by the projects SRDA-0280-12 and DS-2016-0052 from the Slovak Research and Development Agency.

Abstract #77
Eschar development in mice during *Rickettsia parkeri* infection by tick transmission
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Rickettsioses are vector borne diseases that affect humans and a large range of animal species worldwide. New *Rickettsia* species have been recently described as potential human pathogens. *Rickettsia parkeri* is a mild pathogen for humans and has been found in countries of the Western Hemisphere. *Amblyomma maculatum* ticks are considered the main vector of this pathogen in many states within USA. *R. parkeri* induces a febrile illness, with myalgia, malaise, headache, a high incidence of maculopapular eruption, and an eschar at the tick bite area. These eschar lesions maintain the presence of the pathogen, therefore are used as a diagnostic source. We have developed a tick transmission model of *R. parkeri* to study the host immune response with the main objective of evaluating the initial changes in the skin site of tick feeding. We infested C3H/HeN mice with *R. parkeri*-carrying *A. maculatum* nymphs. Skin samples were collected at 24 hours after tick attachment (a.t.a.), and at 6 and 12 days a.t.a.. We evaluated the initial response and development of a skin lesion at the tick site of transmission. Animals infected by infestation with *A. maculatum* carrying the rickettsial pathogen demonstrated bacterial levels at the skin site of infection at 24 hours a.t.a., and 6 and 12 days a.t.a.. Although all organs contained rickettsiae at day 6 a.t.a., inconsistent levels were found between animals at 24 hours a.t.a.. Histological evaluation of the skin revealed an inflammatory response at the tick bite site during attachment; however, no significant differences were observed between infestation with nymphs carrying pathogenic rickettsiae and “uninfected” ticks. Interestingly, at 12 days a.t.a.. the majority of the animals developed a skin lesion characterized by a focus of ulcerative necrotic epidermis, surrounded by an inflammatory infiltration. This eschar was only present in *R. parkeri*-infected animals. Our initial findings are the premise of more detailed studies of the host response at the site of tick inoculation.
Abstract #78
Screening of Ticks – Potential Vectors for Rickettsia in Azerbaijan
Yegana Sultanova¹*, Rita Ismayilova ¹, Sheyda Shikhaliyeva ¹, Huseyn Hajiyev ², Ramil Teymurov ³, Rakif Abdullayev ¹
¹Republican Anti-Plague Station, Azerbaijan; ²Khachmaz Anti-Plague Division; ³Lankaran Anti-Plague Division

The North and the South of Azerbaijan are mountainous rural regions that create favorable environment for ticks and putative tick-borne pathogens. However, their presence has not been systematically investigated. Therefore, the goals of these studies were to attain an overview of the potential vectors for Rickettsia present in Azerbaijan and to determine Rickettsia vectors. Ticks were collected in Northern and Southern regions of Azerbaijan during 2012 - 2017. The tick species were identified, homogenized and RNA was extracted and stored. The collection in the Northern region (Khachmaz, Guba and Gusar) yielded 3,578 ticks belonging to 13 different species. The most common ones were: Rhipicephalus (Boophilus) annulatus (52%) and Arqas periscus (18%). Rickettsia RNA was detected by RT PCR in 58 of the 682 tick pools, all positives belong to Dermacentor marginatus, rendering 8% of the tested tick of this species as vectors. In the Southern region (Lankaran, Masalli and Gizilaghaj National Park) 3,818 ticks of 12 different species were collected. The most common species were: Rhipicephalus (Boophilus) annulatus (71%) and Hyalomma marginatum (21%). Prior study in this region showed presence of Rickettsia felis in Hyalomma spp. The data indicate a broad diversity of tick species in Azerbaijan. Many of the detected tick species could potentially serve as a vector for Rickettsia spp., however so far, Rickettsia RNA was found only in Dermacentor marginatus and Hyalomma spp. Whether these species are indeed the only ones, needs to be confirmed. Additionally, more comprehensive surveillance encompassing all of the country is needed.

Abstract #79
A preliminary comparison of 5 assays for detecting past exposure to Coxella burnetii for use prior to human Q Fever vaccination in Australia.
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²Sullivan Nicolaides Pathology (SNP), Brisbane, Queensland, Australia

Background: Vaccination against Q Fever (Coxiella burnetii infection) is performed in Australia using Q-VAX, a formalin-killed whole cell vaccine. Adverse reactions occur in those persons with prior exposure to C. burnetii. To prevent this adverse reaction pre-vaccination screening is performed and persons found to be positive are excluded from vaccination. Currently a skin test (to detect T-cell immunity) and an antibody assay (to detect B-cell immunity) are undertaken on each person prior to vaccination. However the assay that “best” correlates with prior exposure to C. burnetii and thus adverse reactions to the vaccine is not known. A new interferon-gamma release assay (IGRS), “Q-Detect”, has recently become available. This new assay and 4 current assays were compared. Material/Methods:A small group (n=25) of attendees at an Australian scientific conference were offered Q-VAX vaccination. As part of their pre-vaccination testing, 5 different assays were performed on them to detect their possible prior exposure to C. burnetii. The assays were: 1. intradermal skin test inoculation; 2. serology by immunofluorescence (IF); 3. serology by enzyme immunoassay (EIA); 4. serology by complement fixation test (CFT); 5. IGRA. Results: Of the 25 participants in the study, 7 had prior exposure to C. burnetii due to vaccination against Q Fever and 1 had prior Q Fever infection. The remaining 17 persons had no known prior exposure to C. burnetii. The results from the 5 assays were compared in the 2 groups and the sensitivity and specificity of the assays calculated, as under: (see table).

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<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
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The new IGRA "Q-Detect" is superior to the 4 currently used assays for detecting prior exposure to C. burnetii in persons seeking vaccination against Q Fever in Australia.
**Abstract #80**

Formalin-inactivated *Coxiella burnetii* Phase I Vaccine Elicits Recruitment of Eosinophils to the Spleen  
Lindsey E. Ledbetter* and Guoquan Zhang  
Veterinary Pathobiology, University of Missouri, Columbia, MO 65211

*Coxiella burnetii* (Cb) is an obligate intracellular Gram-negative bacterial pathogen that causes the zoonosis human Q fever. This globally distributed pathogen is an understudied NIH category B priority pathogen for which no FDA-approved vaccine exists. Formalin-inactivated *Cb* phase I vaccine (PIV) significantly reduces splenomegaly and bacterial burden in immunocompetent mice challenged with virulent *Cb* phase I. This protective response can be generated as early as 7 days post-vaccination; however, the mechanism remains unclear. In this study, flow cytometry was used to determine the frequency of immune cell populations in the spleens of aluminum hydroxide adjuvant-treated and PIV-vaccinated BALB/c mice. There were significantly higher frequencies of granulocytes in PIV-vaccinated mice compared to adjuvant-treated controls. This phenotype was observed as early as 1 week post-vaccination and as late as 5 weeks post-vaccination. Surprisingly, we found these infiltrating granulocytes to be SSC−CD11b+CD125+ eosinophils. Eosinophils can play a major role in bridging innate and adaptive immunity by priming B cells and IgM production. It has been demonstrated that PIV elicits protective IgM and IgG, which are sufficient for generating a protective response in immunocompetent mice. These studies provide a potential mechanism of B cell activation in the context of PIV vaccination which requires further investigation.

**Abstract #81**

Both MHC class I and class II molecules are required while MHC-I appears to play a critical role in host defense against primary *Coxiella burnetii* infection  
Laura Buttrum, Lindsey E. Ledbetter*, Rama Cherla, Yan Zhang, William J. Mitchell and Guoquan Zhang  
Veterinary Pathobiology, University of Missouri, Columbia, MO 65211

To understand the role of class I major histocompatibility complex (MHC-I) and class II MHC (MHC-II) antigen presentation pathways in host defense against *C. burnetii* infection, we examined if MHC-I or MHC-II deficiency in mice would significantly influence their susceptibility to virulent *C. burnetii* Nine Mile phase I (NMI) infection. The results indicate that NMI infection induced more severe disease in both MHC-I deficient and MHC-II deficient mice compared to WT mice, while only MHC-I deficient mice developed a severe persistent infection and were unable to control bacterial replication. These results suggest that both MHC-I restricted CD8+ T cells and MHC-II restricted CD4+ T cells contribute to host defense against primary *C. burnetii* infection, while MHC-I restricted CD8+ T cells appear to play a more critical role in controlling bacterial replication. Additionally, although NMI infection induced more severe disease in TAP1 deficient mice than their WT counterparts, TAP1 deficiency in mice did not significantly influence their ability to eliminate *C. burnetii*. This suggests that *C. burnetii* antigen presentation to CD8+ T cells by the MHC-I classical pathway may only partially depend on TAP1. Furthermore, granzyme B deficiency in mice did not significantly alter their susceptibility to *C. burnetii* infection, but perforin deficient mice were unable to control host inflammatory responses during primary *C. burnetii* infection. These results suggest that perforin, but not granzyme B, is required for *C. burnetii* antigen specific cytotoxic CD8+ T cells to control primary *C. burnetii* infection.

**Abstract #82**

Efficacy of Intranasal Immunization with Whole-Cell Fixed *Coxiella burnetii* Nine Mile Phase I on Subsequent Pathogenesis using a Guinea Pig Model  
Edward Shaw  
Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078

*Coxiella burnetii* is an obligate intracellular bacterial pathogen that causes Q fever. The debilitating acute form of the disease is characterized by headache, fever, photophobia, and pneumonia while chronic disease is often associated with valvular endocarditis and hepatitis. *C. burnetii* is environmentally stable, has a low infectious dose, is transmitted by aerosol, and was weaponized in the past. While this pathogen is controlled in the general population by pasteurization, it is on the CDC’s select agent list because of its ability to cause significant morbidity. Studies have shown that
intramuscular, sub-cutaneous, and intraperitoneal administration of whole-cell formalin fixed virulent (phase I) *C. burnetii* vaccine formulations is able to protect subjects from disease following subsequent *C. burnetii* phase I exposure. In an effort to determine if an intranasal route of immunization would elicit a protective immune response to *C. burnetii* infection, we used $10^6$ whole-cell formalin fixed *C. burnetii* Nine Mile phase I (NMI) in combination with a double mutant of *E. coli* heat-labile enterotoxin adjuvant in a guinea pig model. Booster immunizations followed at 14 and 28 days. To determine a general measure of the humoral immune response, IgG titers were assayed by indirect fluorescent antibody microscopy using sera collected prior to, and at 14, 28, and 56 days following intranasal immunization. *Coxiella burnetii* NMI specific titers ranging from 4096 to 16384 were detected in day 56 sera, indicating a robust humoral response was elicited using intranasal immunization. In addition, immunized animals subsequently infected with virulent *C. burnetii* NMI did not develop significant clinical symptoms as measured by weight loss or fever. Studies defining minimal immunization requirements, potential hypersensitivity response, and protective antigen profile using this physiologically relevant exposure route will aid us in defining a protective response to this unusual aerosol acquired pathogen.

**Abstract #83**

*Mechanism of a mimetic peptide vaccine-induced protective immunity against Q fever*

Danielle Freches, Alexandra Elliott, Yan Zhang and Guoquan Zhang*

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO

Q fever is a worldwide zoonotic disease that is caused by the obligate intracellular Gram-negative bacterium, *Coxiella burnetii*. Human Q fever can develop into a severe chronic, potentially fatal disease. Since there is no vaccine commercially available for prevention of human Q fever in the US, creation of a safe and effective new generation vaccine to prevent Q fever remains an important public health goal. Our previous study demonstrated that a peptide mimic of a *C. burnetii* phase I lipopolysaccharide (PI-LPS) epitope (m1E41920) conjugated to keyhole limpet haemocyanin (m1E41920-KLH) conferred significant protection against *C. burnetii* infection in mouse model of Q fever. Our recent study also indicates that m1E41920-KLH was able to provide long-lasting protection against *C. burnetii* infection in mice. These findings provided proof of concept of protective immunity induced with a peptide mimic of a single PI-LPS epitope and support the utility of the mimotope vaccine candidate to prevent human Q fever. In this study, to understand the mechanism of m1E41920-KLH-mediated protection, we examined if m1E41920-KLH-mediated protection depend on antibody and/or T cell-mediated immunity. The results indicate that both immune sera and splenocytes from m1E41920-KLH-vaccinated mice were able to protect nave mice against *C. burnetii* infection but T cells or CD4+ T cells did not provide measurable protection. Additionally, m1E41920-KLH did not elicit a *C. burnetii* antigen-specific T cell response in mice. However, m1E41920-KLH-vaccinated CD4+ T cell deficient mice were unable to confer protection against *C. burnetii* infection. Collectively, these results suggest that m1E41920-KLH-mediated protection depends on antibody-mediated immunity while CD4+ T helper cells are required for generating protective antibody response.

**Abstract #84**

*Designing and evaluating Q fever vaccines in mice and guinea pig models of aerosol infection*

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Q fever is an important zoonotic infection with a global distribution caused by the obligate intracellular bacterium *Coxiella burnetii*. The CDC categorizes *C. burnetii* as a tier 2 biological select agent (BSAT) due to its transmissibility as a highly infectious aerosol and the absence of a safe and effective vaccine. The most successful vaccine to date is a bacterin vaccine made from formalin-inactivated *C. burnetii* phase I Henzerling strain (QVax; Commonwealth Serum Laboratories, Australia), which is able to confer life-long protective immunity in humans after a single dose. However, due to significant safety concerns around vaccinating previously sensitized individuals the vaccine remains unlicensed for use outside of Australia. We employed a dual design strategy for creating next generation vaccine candidates from an attenuated, BSL-2, phase-variant NMI strain. A subunit vaccine approach identified key protective antigens that were screened to evaluate their protective efficacy in a C57Bl/6 mouse model of aerosol *C. burnetii* infection. The most protective antigens were also
tested as conjugates on a gold nanoparticle (AuNP) delivery system, which further reduced splenomegaly and bacterial burden in the lungs and spleen of infected mice. Cellular and serological assays also suggest a role for AuNPs in promoting a Th1 immune response upon vaccination, as demonstrated by elevated IFN-γ levels and immunoglobulin class-switching. The alternate approach used whole cell material (SolWCVII) from NMII that was generated through a variety of membrane solubilization methods. The resulting materials were characterized by mass spectrometry and for the ability to cross-react with infection-derived sera. Subsequent testing of material in murine and guinea pig aerosol challenge models demonstrated the ability to provide levels of protection similar to that of QVax-like material. Moreover, evaluation of reactogenicity of SolWCVII material in a pre-sensitized hairless guinea pig model and demonstrated, that unlike QVax, SolWCVII vaccine material does not produce any significant hypersensitivity.

Abstract #85
Neutrophils and M1 macrophages contribute to vascular injury and lung pathogenesis during Orientia tsutsugamushi infection
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Acute lung injury (ALI) leading to acute respiratory distress syndrome is a prevailing pathologic manifestation during O. tsutsugamushi-induced severe scrub typhus in humans. In this study, we tested a hypothesis that lung pathology in scrub typhus is due in part to dysregulated activation of inflammatory responses, leading to vascular malfunction and ALI. Following infection with a lethal dose of O. tsutsugamushi in mice, lung tissues had a significant increase in ICAM1, VEGFR2, and angiopoietin-2 (Ang2), as measured by flow cytometry and immunofluorescence. Several unique features were also observed. First, a progressive decrease or loss of endothelial functional markers (Ang1, Tie2, and VE-cadherin) and CD41+ platelets, even after the peak of bacterial replication in other infected tissues (around day 6), implying continued endothelial cell stress and tissue damage. Interestingly, CD41+ platelets were seen to colocalize with MPO+ activated leukocytes, an interaction observed in sepsis and during neutrophil extracellular trap (NET) formation and release. Second, a sustained neutrophil influx and activation as disease progressed, and lung-recruited neutrophils became highly activated in releasing azurophilic granules or myeloperoxidase around day 10 (prior to host death). Finally, lung-derived macrophages were highly polarized to an M1 phenotype (CD80+CD64+CD11b+Ly6G+) at D6-D9, with no signs of M2 activation (CD206+CD64+CD11b+Ly6G-). This study reveals specific biomarkers for vascular stress/dysfunction and uncovers a type 1-skewed, but type 2-suppressed, immune responses in the lungs of lethally-infected mice. More importantly, it furthers findings from human patients and cells, implying an immunopathogenic role in ALI development during severe scrub typhus. Understanding of leukocyte effector molecules and endothelial stress pathways triggered by bacterial vs. host factors will help control this neglected tropical disease.

Abstract #86
Developing a Guinea Pig Model to Interrogate the Immune Response to Spotted Fever Rickettsiae
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Immunocompetent mice have limited utility for the study of spotted fever rickettsiosis (SFR) because they do not demonstrate clinical disease. The guinea pig was the original model utilized to evaluate pathogenic potential of spotted fever group Rickettsia species. R.R. Parker postulated the disease potential of Rickettsia parkeri 65 years prior to the first documented human case, using guinea pigs. Furthermore, the human immune system is demonstrably more similar to that of a guinea pig than to mice. Genetic similarities include MHC homology, CD1 proteins, and the complement system. Thus, despite availability of knockout mice and a larger catalog of immunological probes, the guinea pig is a more relevant model for studying the human immune response to SFR. Their large size allows for longitudinal studies requiring blood collections at multiple time points for multiple assays, and CRISPR-Cas9 technology makes guinea pig gene knockouts feasible. Here, we evaluate clinical response to R. parkeri transmitted from Amblyomma maculatum ticks. To advance our understanding of infection in the guinea pig, we also interrogate the adaptive immune response using a six-color flow
cytometric assay. Eschars developed in guinea pigs exposed to one to three A. maculatum that were naturally or artificially infected with R. parkeri; subcutaneous implants to measure temperature demonstrated a fever in one guinea pig. By lymphocyte subset phenotyping, we found that percentages of B and T cells in total lymphocyte populations, percentages of CD4+ and CD8+ lineages in total T cells, and CD4+/CD8+ ratios were comparable between uninfected guinea pigs and normal human reference ranges. Preliminary results indicate possible alterations in these values in infected guinea pigs. Our data suggest that the guinea pig model for tick-transmitted SFR will be more appropriate than mice and ideal for evaluating the immune response. Efforts to add additional markers to our assay and follow additional clinical parameters are underway.

Abstract #87
Restriction Fragment Length Polymorphism Analysis of Orientia tsutsugamushi scaE and scaC Autotransporter Gene Passenger Domains
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Five autotransporter protein genes (called Surface Cell Antigens Sca-A-E) have been described from the genomes of Orientia tsutsugamushi (Ots) by Korean investigators (Ha et al.). We recently identified a fifth sca gene, scaF, in four of the partial Ots genome sequences available at NCBI. By means of specific TaqMan assays designed to amplify conserved regions of the beta barrel transporter domain of the six sca genes, we evaluated the distribution of the genes among 179 isolate DNAs from 12 countries. Only scaB and scaF were not present in many isolates. In order to assess the diversity of scaC and scaE, we have designed conventional PCR primers that amplify the complete passenger domains (PD) of most of these isolates. The sizes of these PD amplicons for these genes was consistent with the data available from the Ots genomic sequences (scaE: 1568-1589 bp, scaC: 839-866 bp); these genes are the smallest of the four widely prevalent sca genes. In order to rapidly identify isolates with highly divergent genotypes of scaE and scaC PD for further full sequence analysis, we have conducted restriction fragment length polymorphism analysis (RFLP) on these amplicons with four restriction enzymes each on 2-3% agarose gels. Some RFLP patterns were easier to score for differences and others were more efficient in identifying multiple genetic types. Groupings of similar isolates and identification of atypical isolates from a particular country was achieved. For example, 2 isolates from China appeared to be unique and differed from each other with both scaC and scaE. On the other hand, only two different types among 7 isolates from New Zealand were identified by scaC typing but 5 types were detected with the larger scaE gene. Whether genetic variation in these surface antigens can affect the clinical manifestations of scrub typhus is unknown.

Abstract #88
A type I interferon/IL-10 axis induced by Orientia tsutsugamushi infection suppresses antigen-specific T cells and their memory responses
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Despite the various roles of type I interferon (type I IFN) responses during bacterial infection, its specific effects in vivo have been poorly characterized in scrub typhus caused by Orientia tsutsugamushi infection. Here, we show that type I IFNs are primarily induced via intracellular nucleic acids sensors, including RIG-I/MAVS and cGAS/STING pathways, during O. tsutsugamushi invasion. However, type I IFN signaling did not significantly affect pathogenesis, mortality, or bacterial burden during primary infection in vivo, when assessed in a mice model lacking a receptor for type I IFNs (IFNAR KO). Rather, it significantly impaired the induction of antigen-specific T cells and reduced memory T cell responses. IFNAR KO mice that recovered from primary infection showed stronger antigen-specific T cell responses, especially Th1 and cytotoxic T lymphocytes, and more efficiently controlled bacteremia during secondary infection than wild type mice. Enhanced IL-10 expression by macrophages in the presence of type I IFN signaling might play a significant role in the suppression of antigen-specific T cell responses as neutralization or knock-out of IL-10 increased T cell responses in vitro. Therefore,
induction of the type I IFN/IL-10 axis by *O. tsutsugamushi* infection might play a significant role in the suppression of T cell responses and contribute to the short longevity of cell-mediated immunity, often observed in scrub typhus patients.

**Abstract #89**

**Activation of ASC Inflammasome Driven by TLR4 Contributes to Host Immunity against *R. australis* Infection**

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Rickettsiae are cytosolic-replicating, obligately intracellular bacteria causing human infections worldwide with potential fatal outcomes. The interactions of rickettsiae with immune events occurring in the cytosol of host cells remain incompletely understood. We previously have shown that ASC plays an essential role in inflammasome activation by *Rickettsia australis* in macrophages. NLRP3 inflammasome only partially contributes to inflammasome activation by *R. australis* in macrophages and host control of these bacteria *in vivo*. In the present study, host susceptibility of ASC inflammasome-deficient mice against *R. australis* was significantly greater than wild type (WT) controls, and was accompanied by increased rickettsial loads in various organs. Impaired host control of *R. australis in vivo* in ASC−/− mice was associated with dramatically reduced levels of IL-1β, IL-18, and IFN-γ in sera. The intracellular concentrations of *R. australis* in bone marrow-derived macrophages (BMMs) of TLR4−/− and ASC−/− mice were significantly greater compared to WT controls, highlighting the important role of these two molecules in controlling rickettsiae in macrophages. Further mechanistic studies suggest that the LPS purified from *R. australis* together with ATP stimulation led to cleavage of inflammasome components including pro-caspase-1, pro-IL-1β and pro-IL-18 resulting in TLR4-dependent secretion of IL-1β. Compared to WT BMMs, TLR4−/− BMMs failed to secrete a significant level of IL-1β and contained reduced expression levels of pro-IL-1β in response to infection with *R. australis*, suggesting that these bacteria activate ASC inflammasome in a TLR4-dependent mechanism. Interestingly, BMMs primed with purified rickettsial LPS followed by *R. australis* infection and ATP stimulation significantly enhanced the secretion levels of IL-1β compared to *R. australis* infection alone. Taken together, our data suggest that activation of ASC inflammasome most likely driven by interaction of TLR4 with rickettsial LPS contributes to host immunity against *R. australis*.

**Abstract #90**

**A novel elncRNA as potential regulator of CD8+ T-cells and anti-rickettsial immunity**

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In-depth understanding of the biology of host-pathogen interactions is important for the development of preventative and treatment countermeasures against infectious diseases; yet the mechanisms underlying regulation of host immune responses to rickettsial infections remain poorly understood. Enhancer long noncoding (elnc) RNA, representing a class of IncRNA transcribed from enhancer loci of the genome, is known to regulate both nearby and distant protein-coding genes in *cis* and *trans*. Through systematic application of RNA-seq, ChIP-seq, and functional genomics analyses on lungs of susceptible mice infected with *R. conorii*, we have identified and analyzed expression kinetics and functions of an active elncRNA NONMMUT013718, providing evidence for its regulation of nearby protein-coding gene Id2 (inhibitor of DNA binding 2). In this study, we investigated the possibility of NONMMUT013718-directed and Id2-mediated activation and clonal expansion of CD8+ T cells during *R. conorii* infection. Our quantitative PCR findings demonstrate significantly increased expression of NONMMUT013718 and its target gene Id2 in *R. conorii*-infected splenocytes and in CD8+ T cells isolated from the spleen of infected mice. Since Id2 has been implicated in determining the fate of CD8+ T cells, we further determined the levels of T cell activation and polarization *in vivo*. Flow cytometry analysis revealed a shift from the CD8+CD27+ T cell subset towards CD8+CD27− subset, as well as clonal expansion of naïve CD8+ (Tn) T cells towards cytotoxic (TEM) and precursor of memory T cells (TeCM), suggesting the selective activation and polarization of cytotoxic T cells during infection. We also observed higher expression of elncRNA and its target gene in CD8+ T cells following co-cultures with *R. conorii*-infected mouse peritoneal macrophages (CRL-2446, as autologous MHC class I antigen-presenting cells). Further,
experiments to investigate the potential beneficial effects of NONMMUT013718 in enhancing CD8+ T cell-mediated immunity during rickettsial infections are therefore warranted and currently ongoing.

**Abstract #91**

**Exchange protein directly activated by cAMP plays a critical role in fatal rickettsioses**

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The prototypic second messenger, cyclic AMP, plays a crucial role in microbial pathogenesis. We found that deletion of the exchange protein directly activated by cyclic AMP gene, EPAC1, in mice protected them from an ordinarily lethal dose of rickettsiae. Inhibition of EPAC1 suppressed bacterial invasion by blocking rickettsial adhering to endothelial surface in a host annexin A2 (ANXA2)-dependent manner. Most importantly, pharmacological inhibition of EPAC1 in vivo using an EPAC-specific small molecule inhibitors (ESIs), completely recapitulated the EPAC1 knockout phenotype. ESIs treatment hampered rickettsial adhering to endothelial apical surface and dramatically decreased the morbidity and mortality associated with fatal spotted fever rickettsiosis. Our results demonstrate that EPAC1-mediated signaling represents a novel mechanism for host-pathogen interactions, and that EPAC1 is a potential therapeutic target for fatal rickettsioses.

**Abstract #92**

**Investigation of *Coxiella burnetii* Phospholipid Metabolism**

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Analysis of the *C. burnetii* genome reveals that many basic metabolic pathways are incomplete. One prominent example is the phospholipid biosynthetic pathway. Phospholipids are integral components of cell membranes, which act as permeability barriers. *C. burnetii* produces an unusual phospholipid profile that is developmentally regulated. Lipid remodeling is mediated by an outer membrane phospholipase A homolog (PldA). Preventing global lipid remodeling by creating a *C. burnetii pldA* null mutant leads to a significant growth defect in THP-1 macrophages. However, assessing the importance of modifications to individual lipids for growth and pathogenesis is complicated by the fact that the pathways used to synthesize each lipid are unknown or incomplete. For example, the phosphatidylglycerol (PG) pathway is missing a homolog to the *pgpB* gene, which codes for a protein required to dephosphorylate phosphatidylglycerolphosphate to produce PG. The goal of this research is to discover and characterize the missing enzyme from the *C. burnetii* PG synthesis pathway. A candidate protein, CBU1267, was identified after comparing enzyme active sites to PgpB, which is a member of the type 2 phosphatidic acid phosphatase (PAP2) family. Subsequent work has focused on CBU1267 purification, development of *in vitro* assays to assess phosphatidylglycerolphosphatase activity and complementation of an *Escherichia coli pgpA/B/C* triple mutant with *cbu1267*.

**Abstract #93**

**Inhibition of mTORC1 by *Coxiella burnetii* promotes replication within a phagolysosome-like vacuole**

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*Coxiella burnetii* is a vacuolar pathogen that colonizes alveolar macrophages. The *Coxiella*-containing vacuole (CCV) traffics canonically through the endosomal pathway culminating with lysosome fusion. Mature CCV label with lysosomal and autophagic markers and harbor active acid hydrolases. Pronounced size and temporal stability differentiate the CCV from a typical phagolysosome. Lysosomal activity is regulated by mammalian (or mechanistic) target of rapamycin (mTOR) complex 1 (mTORC1) kinase in response to nutrient availability. During nutrient depravation, mTORC1 inactivation favors increased lysosomal fusion, autophagic flux, and degradation. Cumulative evidence indicates autophagy components promote CCV homotypic fusion but the involvement of autophagic degradation remains less clear. Here, mTORC1 activity was examined to better understand regulation of lysosomal physiology and cell catabolism during *C. burnetii* infection. We report decreased mTORC1 activity during *C. burnetii* infection occurs in the absence of increased autophagic flux and benefits pathogen replication in host cells. Human macrophages infected with *C. burnetii* exhibited reduced
phosphorylation of mTORC1 substrates 4E-BP1 and p70S6 kinase. Decreased mTOR localization on the CCV and increased nuclear translocation of the lysosomal transcription factor TFE3 during C. burnetii infection provided additional evidence of mTORC1 inhibition. Infected cells contained increased levels of LC3 and p62 but did not exhibit altered autophagic flux relative to control cells. Cells cultured under mTORC1-inhibiting conditions supported larger and more fusogenic CCVs but not increased bacterial replication. By contrast, C. burnetii intracellular growth was impaired in cells with hyperactive mTORC1. These results demonstrate C. burnetii-mediated inhibition of mTORC1 is sufficient for maximal replication. Modulation of mTORC1 is predicted to contribute to CCV adaptation for C. burnetii replication.

Abstract #94
Actin polymerization in the endosomal pathway, but not on the Coxiella-containing vacuole, is essential for pathogen growth
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Coxiella burnetii is an intracellular bacterium that replicates within an expansive phagolysosome-like vacuole. Fusion between the Coxiella-containing vacuole (CCV) and late endosomes/multivesicular bodies requires Rab7, the HOPS tethering complex, and SNARE proteins, with actin also speculated to play a role. Here, we investigated the importance of actin in CCV fusion. Filamentous actin patches formed around the CCV membrane that were preferred sites of vesicular fusion. Accordingly, the mediators of endolysosomal fusion Rab7, VAMP7, and syntaxin 8 were concentrated in CCV actin patches. Generation of actin patches required C. burnetii type 4B secretion and host retromer function. Patches decorated with VPS29 and VPS35, components of the retromer, FAM21 and WASH, members of the WASH complex that engage the retromer, and Arp3, a component of the Arp2/3 complex that generates branched actin filaments. Depletion by siRNA of VPS35 or VPS29 reduced CCV actin patches and caused Rab7 to uniformly distribute in the CCV membrane. C. burnetii grew normally in VPS35 or VPS29 depleted cells, as well as WASH-knockout mouse embryo fibroblasts, where CCV are devoid of actin patches. Endosome recycling to the plasma membrane and trans-Golgi of glucose transporter 1 (GLUT1) and cationic independent-mannose-6-phosphate receptor (CI-M6PR), respectively, was normal in infected cells. However, siRNA knockdown of retromer resulted in aberrant trafficking of GLUT1, but not CI-M6PR, suggesting canonical retrograde trafficking is unaffected by retromer disruption. Treatment with the specific Arp2/3 inhibitor CK-666 strongly inhibited CCV formation, an effect associated with altered endosomal trafficking of transferrin receptor. Collectively, our results show that CCV actin patches generated by retromer, WASH, and Arp2/3 are dispensable for CCV biogenesis and stability. However, Arp2/3-mediated production of actin filaments required for cargo transport within the endosomal system is required for CCV generation. These findings delineate which of the many actin related events that shape the endosomal compartment are important for CCV formation.

Abstract #95
Coxiella burnetii blocks IL-17 signaling in macrophages
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Coxiella burnetii is an obligate intracellular bacterium and the etiological agent of Q fever. Successful host cell infection requires the Type IVB Secretion System (T4BSS), which translocates bacterial effector proteins across the vacuole membrane into the host cytoplasm, where they manipulate a variety of cell processes. To identify host cell targets of Coxiella T4BSS effector proteins, we determined the transcriptome of murine alveolar macrophages infected with a Coxiella T4BSS effector mutant. We identified a set of inflammatory genes that are highly expressed in T4BSS mutant-infected cells compared to mock-infected or cells infected with wild type (WT) bacteria, suggesting Coxiella T4BSS effector proteins downregulate expression of these genes. In addition, the IL-17 signaling pathway was identified as one of the top pathways manipulated by the bacteria. While previous studies demonstrated that IL-17 plays a protective role against several pathogens, the role of IL-17 during Coxiella infection is unknown. We found that IL-17 kills intracellular Coxiella in a dose-dependent manner, with the T4BSS mutant exhibiting significantly more sensitivity to IL-17 than WT bacteria.
Furthermore, we found that the IL-17 bactericidal effect was significantly neutralized by blocking the IL-17 receptor. In addition, quantitative PCR confirmed increased expression of IL-17 downstream genes in T4BSS mutant-infected cells compared to WT or mock-infected cells, including the pro-inflammatory cytokines IL1a, IL1b and TNFα, the chemokines CXCL2/MIP-2 and CCL5/RANTES, and the antimicrobial Lipocalin-2. We further confirmed that CXCL2/MIP-2 and CCL5/RANTES protein levels are downregulated through the Coxiella T4BSS. Together, these data suggest that Coxiella downregulates IL-17 signaling in a T4BSS-dependent manner in order to escape the macrophage immune response.

Abstract #96

In Vitro and In Vivo Localization of the Hypothetical Membrane Protein CBU_1651
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Coxiella burnetii is an obligate intracellular pathogen and the etiological agent of Q-fever. To cause disease, C. burnetii requires a functional Type IVB secretion system (T4BSS) to establish a replicative compartment, termed the parasitophorous vacuole (PV), and from here manipulate host cell functions via the release of effector proteins. A potential effector protein or T4BSS component encoded by cbu_1651, which is unique to C. burnetii, is of interest. Using in silico analyses, CBU_1651 is predicted to contain a secretion signal and a transmembrane domain. Moreover, cbu_1651 is within the icmW and icmX operon and transcriptionally linked, which suggests a possible important function for CBU_1651 during pathogenesis. The objective of this study was to characterize the localization of CBU_1651 during C. burnetii in vitro and in vivo growth. We have developed polyclonal antisera against CBU_1651 for subsequent assays. By immunoblot detection, CBU_1651 was found to be secreted in a T4BSS dependent and lipid mediated manner into the growth medium. During infection, CBU_1651 was observed localizing within the PV lumen and the pole of the bacterial cell. These data suggest that CBU_1651 is mediated by a mechanism that senses environmental stimuli for secretion and potentially associates with the T4BSS for an unknown role during pathogenesis.

Abstract #97

A Tale of New Isolates: Responses of mice to aerosol infection with novel isolates of C. burnetii
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While Coxiella burnetii, the bacteria that causes Q fever, is found in dairy samples across the US, only the genotype ST20 is found in contemporary cow milk samples, and this genotype is not commonly implicated in human infection. In this study we used a mouse aerosol infection model to evaluate the clinical infection and antibody response to isolates in the ST20 genogroup in comparison to 9Mi I, the type strain of Coxiella, which is in the ST16 genogroup, and an additional ST16 isolate. Groups of C57/Bl6 mice were infected with ST20 and ST16 isolates derived from milk, environmental, and human samples via aerosol inoculation. The mice were monitored for weight loss after infection, and had routine blood draws to collect samples for serological testing. A subset of each infected group were euthanized at day 14 and tissue samples tested for the presence of C. burnetii DNA by real-time PCR. The remaining animals were euthanized 12 weeks post-infection and tissue samples also tested by real-time PCR. Serum samples were tested by an IgG and IgM immunofluorescence assay in order to determine serological response over time. Weight loss and splenomegaly were less severe in both ST20 isolates and the low-passage ST16 isolate compared to 9Mi I. In addition, antibody response rates were delayed in the ST20 and ST16 isolates relative to 9Mi I. The data suggest that novel ST20 and ST16 isolates have reduced virulence compared to 9Mi I in this model, but differences could relate to passage history rather than inherent genetic differences.
Abstract #98
The Coxiella burnetii Type IV Effector CBU0794 Modulates Host Nuclear Processes
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Coxiella burnetii is a Gram negative, obligate intracellular pathogen and the etiological agent of the zoonotic disease known as Q fever. This organism utilizes an essential type IVB secretion system (T4BSS) to promote its intracellular survival and replication, but the majority of effectors have not been well characterized. In a large-scale screen of T4BSS substrates, we previously showed six predicted open reading frames (ORFs) which code for proteins that exhibited nuclear localization when expressed ectopically in Hela cells. One of these ORFs, CBU0794, contained two potential nuclear localization signals (NLSs) based on bioinformatics analysis. We tested the functionality of these potential NLSs by making specific deletions and comparing the impact on their nuclear localization patterns to find that one NLS was sufficient for nuclear translocation. An Epistasis Miniarray profile (E-MAP) screen in S. cerevisiae, which provides a global quantitative genetic profile of host interactions, was used to predict the function of CBU0794 in host cells. This screen predicted CBU0794 is involved in regulation of host gene transcription. This interaction was confirmed in mammalian cells using protein pulldowns, which showed an interaction with the host nuclear protein TBLR1, a key member of the SMRT/NCor-HDAC3 complex involved in transcriptional repression. Our results reveal a previously uncharacterized interaction of a C. burnetii nucleomodulin with host nuclear transcription factors.

Abstract #99
Ehrlichia chaffeensis TRP120 HECT Ub Ligase Targets FBW7 for Degradation to Maintain NOTCH Signaling to Promote Infection
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Human monocytotropic ehrlichiosis (HME) is an emerging tick-borne zoonosis caused by the obligately intracellular, gram-negative bacterium, Ehrlichia chaffeensis. E. chaffeensis survival is dependent on secreted tandem-repeat effector proteins (TRPs), which function to manipulate host cellular processes such as cell proliferation and differentiation. The secreted TRP120 has multiple functions and recently has been shown to function as a HECT-like E3 ligase. Using yeast two-hybrid assay, we determined that TRP120 interacts with FBW7 (F-box and WD domain repeat-containing 7), the substrate recognition subunit of the human E3 ligase complex SCF (Skp1, Cul1, and F-box). FBW7 functions as a tumor suppressor, targeting many oncoproteins such as Notch, cyclin E1, MYC, and JUN. Also, we have shown that knockdown of FBW7 leads to increased ehrlichial infection, suggesting that regulation of FBW7 is crucial for maintaining infection. The purpose of this study is to define the TRP120-interacting domains of FBW7 and demonstrate TRP120 ubiquitinates FBW7 for degradation to maintain Notch signaling. We determine that FBW7 levels are decreased in E. chaffeensis-infected cells compared to controls by quantitative immunofluorescent microscopy and Western immunoblotting. Using ectopic expression, we determined TRP120 interacts with FBW7 F-Box and WD40 domains. In addition, we demonstrated that TRP120 directly ubiquitinates FBW7 both in vivo and in vitro with K48-linkage chains, a type of polyubiquitination that targets substrates for proteasomal degradation. Lastly, we observed a significant upregulation of Notch signaling during infection, as a result of FBW7 degradation. These findings support the hypothesis that E. chaffeensis TRP120 effector negatively regulates FBW7 stability through ubiquitination-driven degradation, thereby maintaining Notch signaling to promote intracellular infection. Future studies will investigate the effect of the interaction between TRP120 and FBW7 on cMyc signaling, and its role in apoptosis regulation during Ehrlichia infection.

Abstract #100
Characterization of the eukaryotic Wnt ligand mimic properties of Ehrlichia chaffeensis effector TRP120
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Ehrlichia chaffeensis (E. ch.) is a gram-negative, obligately intracellular pathogen that primarily infects monocytes. Entry of the bacterium into the host cell is dependent on ehrlichial surface-expressed tandem repeat protein (TRP) 120 which
functions as an adhesin and is sufficient for induction of phagocytosis in monocytes. However, a cognate host receptor has not been identified. Our lab previously reported that as early as one hour post E. ch. infection of THP-1 monocytes, the Wnt signal cascade is activated in the host cell, and inhibitors against this pathway significantly reduce infection. Interestingly, TRP120-induced phagocytosis can be blocked by the same inhibitors, suggesting the Wnt receptor complex may act as a receptor for TRP120 through which the signal cascade is activated during infection. Wnt signaling is a conserved eukaryotic signaling pathway that regulates a multitude of cellular events from cell fate determination to innate immunity and is initiated by the binding of a Wnt ligand to a Frizzled (Fzd) receptor. In this study, we investigated the hypothesis that TRP120 utilizes Wnt ligand mimicry to activate the pathway and establish ehrlichial intracellular infection. We used in silico approaches to predict functional similarity between TRP120 and various Wnt ligands and showed in vitro that recombinant TRP120 induces Wnt pathway activity. Using immunofluorescence microscopy, we demonstrated that both E. ch. whole bacterium and recombinant TRP120 colocalize with the Wnt receptor Fzd5. We also immunoprecipitated Fzd5 and TRP120 from infected cell lysate, implying interaction between the proteins during infection. Through these studies, we aim to demonstrate that E. ch. hijacks Wnt signaling in the host cell through molecular mimicry of Wnt ligands.

**Abstract #101**

Interaction of Apoptosis and Actin Cytoskeleton-Associated Host Proteins with *Ehrlichia chaffeensis* TRP75 Promotes Infection

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*Ehrlichia chaffeensis* is an obligately intracellular bacterium that exhibits tropism for mononuclear phagocytes and exploits host cell processes to subvert innate defenses for infection. During infection, ehrlichial secreted effector proteins including a subset of tandem repeat proteins (TRP) are crucial, but the mechanisms involved are still not clear. By yeast two-hybrid analysis, this study revealed that consistent with other TRPs, *E. chaffeensis* TRP75 interacts with a large group of human proteins involved in various biological processes of the host cell, including homeostasis, metabolism, actin cytoskeleton reorganization and apoptosis. Thirteen identified host target proteins were selected and confirmed to interact with TRP75 by co-immunoprecipitation assays. These proteins were further examined by immunofluorescent confocal microscopy during infection and found to colocalize with *Ehrlichia* morulae with different intensities. Moreover, 86 identified TRP75-interacting host proteins were targeted by siRNA separately and we found by real-time qPCR that knockdown of 74 (86%) TRP75 target proteins had significant negative influence on ehrlichial infection. This investigation further demonstrates that *Ehrlichia* TRPs interact with many host proteins important for infection.

**Abstract #102**

A genetic system for creating targeted mutations to disrupt and restore genes in *Ehrlichia chaffeensis* that is broadly applicable to other obligate bacteria

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Obligate intracellular bacteria (obligates) belonging to the order Rickettsiales and Chlamydiales are responsible for causing diseases in hundreds of millions of people worldwide. Lack of an efficient system for targeted mutagenesis in obligates remains a major impediment in understanding microbial pathogenesis and in defining the functional significance of many genes. Challenges in creating targeted mutations may be attributed to the essential nature of a gene selected for mutagenesis, intracellular replication dependence and the lack of methods to support extracellular growth. Despite the success in generating many random mutations using transposon mutagenesis, and having a limited success of creating targeted mutations in rickettsial and chlamydial pathogens, presently a method that works well in creating targeted mutations in specific genes of interest followed by complementation remains problematic for the obligate pathogens and is also a highly sought-after goal. We have filled this major methodological deficiency by developing protocols to generate stable targeted mutations by allelic exchange method in *Ehrlichia chaffeensis*, an obligate intracellular tick-borne bacterium responsible for the disease, human monocytic ehrlichiosis. Targeted mutations in *E. chaffeensis* were created to not only to disrupt two genes, but also to restore the intact gene by another allelic exchange mutation, which resulted
in the restored transcription from the inactivated gene from its own promoter. We expect that the methods developed are broadly applicable to other obligate intracellular bacteria to routinely perform targeted mutations to enable studies focused on structure-function analyses of bacterial proteins, host-pathogen interactions and in developing vaccines.

**Abstract #103**

**Sequence Determinants Spanning -10 Motif and Spacer Region Impacting Ehrlichia chaffeensis Sigma 32-Dependent Dnak Gene Promoter Activity**

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*Ehrlichia chaffeensis* is an obligate intracellular tick-borne bacterium, which causes the disease, human monocytic ehrlichiosis. *E. chaffeensis* contains only two sigma factors, σ32 and σ70. It is difficult to study gene regulation in this pathogen and in related rickettsials, as they do not naturally harbor plasmids. We developed an *E. coli*-based transcription system to study *E. chaffeensis* transcriptional regulation. An *E. coli* strain where its chromosomal σ32 was inactivated is used to express *E. chaffeensis* σ32 protein. The *E. coli* system and our previously established *in vitro* transcription system were then used to map the promoter of a σ32-dependent *dnak* promoter by performing detailed mutational analysis. Deletion of -10 or -35 motif of *dnak* promoter resulted in reducing the promoter activity. Similarly, point mutations at all six positions within the -10 motif caused variable impact on the promoter activity. Sequence and length of spacer also played a role in altering the gene activity. We further mapped region 1, 2 and 3 of σ32 for their role in the promoter activity of *dnak*. This is the first study defining the promoter region of a σ32-dependent gene of *E. chaffeensis*.

**Abstract #104**

**Differential Susceptibility of Laboratory Mice to A. phagocytophilum Infection**

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Human granulocytic anaplasmosis (HGA) is an emerging tick-transmitted infection caused by *Anaplasma phagocytophilum*. The non-specific febrile illness carries the risk of fatality in the elderly and immunocompromised. Epidemiological and disease surveillance studies indicate a higher prevalence of HGA in males compared with females with one study citing as high as a 3:1 male predominance. Whether this disparity correlates with differential susceptibility of males versus females to *A. phagocytophilum* infection is unknown. Laboratory mice have been used as models to study granulocytic anaplasmosis for more than two decades. Yet, to our knowledge, sex as a biological variable (SABV) in this model has not been evaluated. Herein, groups of male and female C57Bl/6 mice were intraperitoneally inoculated with *A. phagocytophilum*. Bacterial load in the peripheral blood was monitored at several post infection time points using quantitative PCR and by microscopic examination of peripheral blood smears to determine the percentage of infected cells harboring *A. phagocytophilum* morulae (inclusions). Both male and female mice were susceptible to infection. However, the bacterial load in the peripheral blood of males was higher than that of females, as demonstrated by as much as a 2.5-fold increase in the number of neutrophils bearing morulae and up to a 3.9-fold increase in the relative amount *A. phagocytophilum* 16S DNA at peak infection. The propensity of male mice to develop a higher level of *A. phagocytophilum* infection is relevant for studies utilizing the mouse model, stresses the importance of including SABV, and agree with the observed higher incidence of infection in male versus female patients.

**Abstract #105**

**The cell wall of Orientia tsutsugamushi and other Rickettsiales species**

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Bacterial cell walls consist of a thick polymer structure of sugars crosslinked by amino acid bridges called peptidoglycan, which is essential for their cell shape and protection from osmotic stress. The fact that it is essential, and unique to bacteria, means that eukaryotic cells have evolved to recognize it as a highly immunostimulatory PAMP, and it is also an
important antibiotic target. Obligate intracellular bacteria have evolved so that they are able to replicate exclusively inside host cells, and the resulting proximity to peptidoglycan receptors may cause selective pressure to reduce the amount of immunostimulatory peptidoglycan in their cell wall. There are two major groups of obligate intracellular bacteria: Chlamydiales and Rickettsiales. The peptidoglycan structure in the Chlamydiales has been more extensively studied. Here we show that Orientia tsutsugamushi, a neglected pathogen of the Rickettsiales group, does possess a peptidoglycan-like structure in its cell wall, contrary to previous reports. Orientia also has an outer membrane comprising of a network of disulfide crosslinked proteins, and this works together with the peptidoglycan-like structure to confer some structural rigidity and osmotic protection. The minimal peptidoglycan-like structure of Orientia presents similarities with that of the Chlamydiales group, indicating convergent evolution to pressures of the obligate intracellular lifestyle.

Abstract #106
Inactivation of host EPAC1 can prevent spotted fever group rickettsia from adhering to vascular luminal surface
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Rickettsioses represent some of the most devastating human infections. It has been forecasted that temperature increases due to global climate change will lead to more widespread incidence of rickettsioses. Although rickettsial infections can be controlled by antibiotic therapy if diagnosed early, a 32% fatality has been reported in hospitalized patients of SFGR. A vaccine is not available for fatal rickettsioses, and novel treatments are urgently needed. cAMP-based cell signaling mediated by intracellular cAMP receptors, EPAC1 and 2, are major contributors to the transduction of the effects of cAMP. We have already shown that genetic abolition of host EPAC1 protected mice from fatal rickettsioses. Most importantly, pharmacological inhibition of EPAC using small-molecule EPAC-specific inhibitors (ESIs) in vivo recapitulates the EPAC1-null phenotype: wild-type mice treated with an ESI are protected from fatal rickettsioses. Here, taking advantage of a novel anatomy-based in vivo quantitative bacterial adhesion measuring system, we report that global depletion of EPAC1 blocked rickettsial adherence to the blood vessel luminal surface. Furthermore, functional imaging of living endothelial cell (EC) surface using atomic force microscopy (AFM) demonstrated that ESI09 treatment directly weakened the nanoforces of the recombinant rickettsial Omp8-living EC surface interactions. Our results reveal that EPAC1-mediated signaling axis plays critical role in regulating the rickettsial adhesion.

Abstract #107
Molecular, biochemical and cellular characterization of clinic isolates of Rickettsia sp
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The clinical variations among the rickettsioses cases registered in the same geographic zone, caused by the same species, could due to different virulence or pathogenicity of potential strains of the bacteria; or to different immune response and physic conditions of the patients. The analysis of clinical isolates is an approach to study the first possibility and discard the patients influence, to further study the pathogenic differences among isolates which could explain the differential infection severity in order to obtain vaccine or diagnosis candidates. In this work we obtained isolates of Rickettsia sp. from patients with differential clinical severity through infection of Vero cells in 16-well plates. Isolates were established after 3 passages, and its molecular characterization was obtained by sequencing of OmpB and 17kDa genes. Infectivity was obtained through infection of Vero cells with standardized amounts of bacteria established by qPCR. Cellular damage capabilities will be established by ongoing biochemical assays. At this moment, we have obtained 10 different isolates that are positive to Rickettsia typhi. 4 of these isolates are considered of high virulence (% infected cells >50% after 30'; release to media of > 500 U/ml LDH, > 7 U/ml GSh, > 400 U/ml H2O2) and have a direct correlation with clinical characteristics of
the patients. This strategy will allow us to obtain isolates, that are suitable of analysis through different approaches in order to identify proteins that are involved in its differential pathogenicity.

**Abstract #108**

**MicroRNA-regulated rickettsial invasion into host endothelium via Fibroblast Growth Factor (FGF)-2 and its receptor FGFR-1**

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Microvascular endothelial cells represent the primary target cells during human rickettsioses and respond to infection via activation of immediate-early signaling cascades and resultant induction of gene expression. As small non-coding RNAs dispersed throughout the genome, microRNAs (miRs) regulate gene expression post-transcriptionally to govern a wide range of biological processes. Based on our published findings of the involvement of Fibroblast Growth Factor-2 (FGF-2) and its receptor Fibroblast Growth Factor Receptor-1 (FGFR-1) in facilitating rickettsial invasion into host cells and a recently published study suggesting miR424 and miR503 as regulators of FGF-2/FGFR-1 in microvascular endothelium, we measured the expression of miR424 and miR503 during *R. conorii* infection of human microvascular endothelial cells (ECs). Our results revealed a significant decrease in miR424 and miR503 expression in apparent correlation with increased expression of both FGF-2 and FGFR-1. Taking into consideration the established phenomenon of endothelial heterogeneity, pulmonary and cerebral edema as the prominent pathogenic features of rickettsial infections, and significant pathogen burden in the lungs and brain as target organ systems in established mouse models of disease, we next quantified miR424 and miR503 expression in pulmonary and cerebral microvascular ECs as well. Again, *R. conorii* infection dramatically down-regulated both miRs in these tissue-specific ECs as early as 30 minutes post-infection in correlation with higher FGF-2/FGFR-1 expression as compared to the corresponding uninfected controls. Further, transfection of a miR424 mimic reduced the expression of FGF-2/FGFR-1 and a corresponding decrease in *R. conorii* invasion into host ECs, while an inhibitor of miR424 had the expected opposite effect. Together, these findings implicate rickettsial manipulation of host gene expression via regulatory miRs to ensure efficient entry as a critical requirement for intracellular infection.

**Abstract #109**

**Anaplasma-phagocytophilum–related defects in CD8, NKT, and NK lymphocyte cytotoxicity**

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Human granulocytic anaplasmosis (HGA), caused by the tick-transmitted *Anaplasma phagocytophilum*, is not controlled by innate immunity, and induces a proinflammatory disease state with innate immune cell activation. In *A. phagocytophilum* murine infection models, hepatic injury occurs with production of IFNγ thought to be derived from NK, NKT cells, and CD8 T lymphocytes. Specific *A. phagocytophilum* ligands that drive inflammation and disease are not known but suggest a clinical and pathophysiological basis strikingly like macrophage activation syndrome (MAS) and hemophagocytic syndrome (HPS). We studied in vivo responses of NK, NKT, and CD8 T lymphocytes from infected animals for correlates of lymphocyte-mediated cytotoxicity and examined in vitro interactions with *A. phagocytophilum*-loaded antigen presenting cells (APCs). Murine splenocytes were examined and found deficient in cytotoxicity as determined by CD107a expression in vitro for specific CTL effector subsets as determined by flow cytometry. Moreover, *A. phagocytophilum*-loaded APCs did not lead to IFNγ production among CTLs in vitro. These findings support the concept of impaired cytotoxicity with *A. phagocytophilum* presentation by APCs that express MHC class I and that interact with innate and adaptive immune cells with or after infection. The findings strengthen the concept of an enhanced proinflammatory phenotype like MAS and HPS disease states as the basis of disease and severity with *A. phagocytophilum* infection, and perhaps by other obligate intracellular bacteria.
Abstract #110

Human anti-OMP-1 monoclonal antibody inhibits *Ehrlichia chaffeensis* through TRIM21 mediated autophagy
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*Ehrlichia chaffeensis* is a gram-negative, obligately intracellular bacterium and etiologic agent of human monocytotropic ehrlichiosis (HME), an emerging, life-threatening, tick-borne zoonosis. It is established that antibody mediated immunity is essential for protection against *E. chaffeensis* infection. Humoral immunity to *E. chaffeensis* occurs, at least in part, during the extracellular stage by blocking attachment or via FcyR-dependent mechanisms. We determined that antibodies are also protective through intracellular mechanisms and recently found a non-entry blocking human monoclonal antibody specific to *E. chaffeensis* outer membrane protein (anti-OMP-1 huMab), that restricts ehrlichial growth by engaging the intracellular FcR tripartite motif 21 (TRIM21). *E. chaffeensis* colocalized with cytosolic FcR TRIM21 only in the presence of anti-OMP-1 huMab as observed by confocal microscopy, with a corresponding increase in proinflammatory cytokines and chemokines. Significant reduction in ehrlichial infection was observed in cells treated with anti-OMP-1 antibody. This antibody-mediated intracellular restriction was significantly abrogated by genetic deletion of TRIM21 in THP-1 cells. Recently, it has been determined that some TRIM proteins are involved in a specialized form of autophagy by acting as receptors for specific cargo, and as regulators of autophagy. Hence, we investigated the role of TRIM21 in the autophagic clearance of ehrlichiae in the presence of anti-OMP-1 huMab. *E. chaffeensis* replicates in vacuoles, which fuse with autophagosomes to form amphisomes for nutrient acquisition, but actively evades fusion with lysosomes during replication. In the presence of huMabs, we observed colocalization of ehrlichiae and TRIM21 with polyubiquitin chains, key autophagy regulators (ULK1, Beclin 1), autophagy receptors (LC3, p62) and the lysosomal marker LAMP2. Further, autophagic flux was observed by Western blot, showing the conversion of LC3I to LC3II, accumulation and degradation of p62, and degradation of ehrlichial OMP-1, in the presence of huMab. Our data demonstrates selective autophagic degradation of *E. chaffeensis* as mediated by antibody-dependent recruitment of TRIM21.

Abstract #111

Immunization with a recombinant antigen composed of conserved blocks from TSA56 provides broad genotype protection against Scrub typhus
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Scrub typhus is an acute febrile disease caused by *Orientia tsutsugamushi* infection. Recently, the rapid increase of scrub typhus incidence in several countries within endemic region has become a serious public health issue. Despite the wide range of preventative approaches that have been attempted in the past 70 years, all have failed to develop an effective prophylactic vaccine. Currently, the selection of the proper antigens is one of the critical barriers to generating cross-protective immunity against antigenically-variable strains of *O. tsutsugamushi*. In this study, we defined conserved and variable blocks of TSA56 protein, a highly variable and major outer membrane antigen of *O. tsutsugamushi*, using 206 sequences available in Genbank database and evaluated the protective attributes of a novel recombinant antigen (cTSA56) composed of conserved blocks from TSA56 in lethal *O. tsutsugamushi* infection in mice. Our findings demonstrate that immunization of cTSA56 induces consistent T cell responses, including antigen-specific CD4 and CD8 T cells against diverse TSA56 antigens derived from various genotypes. In addition, cTSA56 vaccination confers significantly enhanced protection against various *O. tsutsugamushi* strains. Interestingly, majority of T cell epitopes predicted by bioinformatics analysis is located within the conserved blocks of TSA56. Therefore, recombinant cTSA56 protein could be used as a novel vaccine antigen for scrub typhus.
Abstract #112
Evaluation of T-cell population cytokine signatures associated with a sub-lethal mouse model of Orientia tsutsugamushi and comparison to lethal and non-lethal mouse models of O. tsutsugamushi
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The immunogenic diversity of Orientia tsutsugamushi as well as the inter-strain difference(s) associated with virulence in mice impose the necessity to dissect the host immune response. In a previous study, we compared the host response in lethal and non-lethal murine models of O. tsutsugamushi infection using the two strains, Karp (New Guinea) and Woods (Australia). Multicolor flow cytometry was utilized to analyze the CD4+ and CD8+ T cell populations, their intracellular production of the cytokines IFNγ, TNF, and IL2, and to identify the specific cytokine signatures associated with our lethal and non-lethal murine models of infection. Correspondingly, we applied the same methodology to analyze the T-cell populations associated with our sub-lethal murine model of O. tsutsugamushi (Gilliam (Burma)), in an effort to determine the exclusivity of T-cell cytokine populations associated with each model (lethal, sub-lethal, and non-lethal). Intra- and inter-strain comparisons revealed distinct, as well as shared, T-cell cytokine populations associated with each model. In addition, we monitored bacterial trafficking to the liver, lung, spleen, kidney, heart, and blood, and seroconversion during each 21-day challenge. Lethal and non-lethal model cytokine signatures are currently being assessed as depletion targets in follow-on experiments to confirm their association with virulence and/or protection.

Abstract #113
Rickettsia rickettsii whole cell antigen vaccine offers protection against virulent pathogen infection challenge
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Rocky Mountain Spotted Fever (RMSF) is a potentially fatal tick-borne disease in people and dogs. RMSF is reported in the USA and other countries in South America. The disease causing agent, Rickettsia rickettsii, is transmitted by several species of ticks in the United States, including Dermacentor andersoni, Rhipicephalus sanguineus, and Amblyomma americanum. Clinical signs following the infection generally include fever, headache, nausea, vomiting, muscle pain, lack of appetite, and rash. If untreated, the disease can quickly escalate into a life-threatening illness in people and dogs. While RMSF is known for over a century, recent epidemiological data suggest that the documented cases of infection in people are on the rise during the last two decades. As of now, there are no vaccines available to prevent R. rickettsii infection in either dogs or people. In this study, we have investigated the efficacy of two approaches to vaccination, based on recombinant immunodominant outer membrane protein antigens (RCA) or whole cell inactivated antigens (WCA), in conferring protection against virulent R. rickettsii infection challenge in the canine host. Our data suggest that dogs vaccinated with the WCA are protected from RMSF, whereas those receiving RCA develop the disease similar to non-vaccinated R. rickettsii-infected dogs. The WCA vaccine is able to reduce rickettsemia and induce an antigen-specific immune response. This study provides the first evidence of vaccine-associated protection against RMSF in a newly developed canine host model of infection.
Abstract #114
Understanding the significance of the interactions between Spotted Fever Group Rickettsia and mammalian phagocytic cells
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Members of the spotted fever group (SFG) Rickettsia are obligate intracellular Gram-negative bacteria that are typically transmitted to a mammal during the blood meal acquisition of a feeding tick. While endothelial cells have long been considered the main target cells for rickettsiae during initial stages of infection, several studies have demonstrated parasitism of circulating monocytes, resident macrophages, neutrophils, lymphocytes and hepatocytes in vitro and in vivo by various pathogenic rickettsial species. Interestingly, our group recently reported that two SFG Rickettsia, R. Rickettsia and R. montanensis, (a species not associated with disease in mammals) are both able to proliferate within non-phagocytic mammalian cell lines. However, these two rickettsial species have completely distinct intracellular fates in human THP-1-derived macrophages (Curto P. et al. (2016) Front. Cell. Infect. Microbiol. 6:80). Numerous intact R. conorii cells can be found in the cytoplasm of PMA-differentiated THP-1 cells and are able to effectively grow within these macrophage-like cells similarly to growth kinetics observed in endothelial (Eahy.926) and epithelial (Vero) cell lines. In sharp contrast, R. montanensis cells are rapidly destroyed within differentiated THP-1 cells in compartments resembling a phago-lysosome. These studies suggested that the ability of Rickettsia species to cause disease in mammals may be correlated in part with the ability of a particular species to proliferate in phagocytes. To further test this hypothesis, we performed growth studies of a panel of Rickettsia species including known human pathogens (R. australis, R. sibirica, R. rickettsii, R. akari) and species not associated with human disease (R. amblyomatis and R. bellii) using PMA-differentiated THP-1 cells and the Eahy.926 human endothelial-like cell line. As was previously reported for R. conorii, SFG Rickettsia species that are associated with human disease were able to efficiently proliferate within non-phagocytic and phagocytic human cell lines. In contrast, Rickettsia species not generally associated with recognized diseases in mammals were able to proliferate within endothelial-like cells, but were unable to effectively grow within THP-1 macrophage-like cells. Preliminary comparative genomic studies of these species revealed the presence of cohorts of genes in the recognized pathogens that are either absent or disrupted in so-called “non-pathogenic” species of Rickettsia. Together, these results, and our ongoing work may provide for the development of a phenotypic diagnostic tool to assess the pathogenic potential of new Rickettsia isolates and species. More importantly, these studies may shed insight into the genetic determinants of Rickettsia pathogenicity in mammals and the strategies that pathogenic members of this genus utilize to disseminate within the infected mammalian host.

Abstract #115
Identification of Type IV Secretion Effectors that are Specifically Required for Replication of Coxiella burnetii in Primary Macrophages
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Coxiella burnetii is a Gram negative, obligate intracellular pathogen and the etiologic agent of acute and chronic Q fever. The organism employs an essential type IVB secretion system (T4SS) to promote intracellular survival, replication and virulence. Using transposon mutagenesis of Nine Mile Phase II (NMII), we determined that while several of these T4SS substrates appear to be required for replication in eukaryotic cell lines, others are dispensable for Coxiella-containing vacuole (CCV) formation and replication. Indeed, approximately half of identified T4SS effectors have been reported in the literature as having no discernable growth defect upon genetic disruption. In an effort to define the contribution of these apparently nonessential effectors to Coxiella virulence, we employed a C57/B6 bone marrow derived macrophage (BMDM) model of infection to determine if the effectors had a cell-specific phenotype. We established this infection model in an effort to study Coxiella in a primary macrophage, which represents an especially relevant pathogenic niche for the organism. In our BMDM infection model, NMII replicates with kinetics comparable to those observed in other traditional eukaryotic cell lines (Vero, HeLa, J774.A1). With regard to T4SS mutants, we found that while none of the strains we tested had a discernable defect in CCV biogenesis in immortalized cells, they were specifically required for replication in BMDMs.
Among these screened mutants, we found three distinct growth phenotypes within BMDM: those that are capable of replication comparable to wildtype, those that can survive, but do not replicate within BMDMs, and those that are readily killed. For those with BMDM-specific attenuation, we confirmed the phenotypes in a SCID mouse model of acute disease. Experiments are ongoing to determine the molecular contribution of each effector to Coxiella burnetii pathogenesis.

**Abstract #116**

**An Ehrlichia type IV secretion system effector Etf-2 binds to active RAB5, and delays endosome maturation**

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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. How RAB5 is sequestered on ehrlichial inclusions is poorly understood, however. We found that native Ehrlichia translocated factor-2 (Etf-2), a previously predicted effector of the *Ehrlichia* type IV secretion system, and recombinant Etf-2 (cloned into the *Ehrlichia* genome) are secreted into the host cell cytoplasm and localize to ehrlichial inclusions. Ectopically expressed Etf-2-GFP also localized to inclusions and membranes of early endosomes marked with RAB5 and interacted with constitutively active RAB5 but not with a dominant-negative RAB5. Etf-2, although lacking a RAB GTPase-activating protein (GAP) Tre2-Bub2-Cdc16 (TBC) domain, contains two conserved TBC domain motifs, namely an Arg finger and a Gln finger, and site-directed mutagenesis revealed that these motifs are required for Etf-2 localization to early endosomes. The yeast two-hybrid assay and microscale thermophoresis revealed that Etf-2 binds tightly to GTP-bound RAB5 but not GDP-bound RAB5. However, Etf-2 lacks RAB5-specific GAP (RABGAP5) activity. Etf-2 localized to bead-containing phagosomes as well as endosomes containing beads coated with the C-terminal fragment of EtpE “entry-triggering protein of Ehrlichia”, an *Ehrlichia* outer-membrane invasin and significantly delayed RAB5 dissociation from, and RAB7 localization to, phagosomes/endosomes and RABGAP5 localization to endosomes. Thus, binding of Etf-2 to RAB5-GTP appears to delay RAB5 inactivation by impeding RABGAP5 localization to endosomes. This is the first example of a bacterial effector that blocks endosomal maturation by binding to RAB5-GTP to benefit bacterial replication and survival.

**Abstract #117**

**Acid Sphingomyelinase is Essential for the Infection Cycles of Vacuole Adapted Pathogens**

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Obligate intracellular bacteria are significant causes of morbidity and mortality with over two hundred and fifty million infections worldwide annually. In order to complete their infection cycles, they must subvert host defense mechanisms and parasitize trafficking pathways. Acid sphingomyelinase (ASMase) is a lysosomal enzyme that is essential for diverse cellular processes including endolysosomal cholesterol transport and generation of ceramide through sphingomyelin hydrolysis. We discovered that ASMase is essential for survival and completion of the infection cycles of the vacuole adapted pathogens: *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), *Coxiella burnetti* (Q fever), and *Chlamydia* spp. (STD, infectious blindness, pneumonia). By first studying *A. phagocytophilum*, we found that tricyclic antidepressants (TCAs), FDA-approved drugs that inhibit ASMase, arrest the bacterium’s infection cycle in a dose-dependent and reversible manner. TCAs inhibit vacuole maturation and expansion, prevent conversion from the replicative form to the infectious form, and eliminate the production of infectious progeny. Similar to the *in vitro* model, *A. phagocytophilum* cannot establish a productive infection in both ASMase²⁻ mice and TCA treated wild-type mice. TCA treatment induces an accumulation of cholesterol within lysosomes and has a rapid bacteriocidal effect on *C. burnetti* within host cells. Additionally, TCA treatment inhibits *C. trachomatis* and *C. pneumoniae* inclusion expansion and infectious progeny generation, with *C. pneumoniae* being more severely impacted. These data highlight the critical, yet distinct roles that ASMase plays in these pathogens’ infection cycles. Furthermore, these results signify the therapeutic potential of TCAs for treating diseases caused by these pathogens.
Abstract #118

Nuclear import of *Anaplasma phagocytophilum* AnkA uses a eukaryotic code in N-terminal ankyrin repeats known to bind RanGDP

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*Anaplasma phagocytophilum* is an obligate intracellular bacterium that causes tick-borne human granulocytic anaplasmosis. The bacterium occupies a modified vacuole within mammalian neutrophils as part of its life cycle. It utilizes Type 4 secretion to inject proteins that facilitate bacterial survival and growth inside the host cells. We showed previously that an injected protein, Ankyrin A (AnkA), enters the nucleus and modifies transcription and likely guides reorganization of chromatin structure. AnkA belongs to a class of proteins containing ankyrin repeat (AR) motifs important for protein-protein and protein-DNA interactions. Previously, we showed that deletion of the N-terminal 4 ARs inhibited nuclear localization, and others showed that in mammalian AR-containing proteins, nuclear localization is dependent upon 2 consecutive ARs where the 13th residues are hydrophobic. Here, we investigated whether single or dual mutations among the N-terminal 4 ARs of AnkA-GFP affect nuclear transport of AnkA in HEK293T cells, and whether this is dependent upon β-importin. Point mutations were made to change the single residues from AnkA_AR1.R56A, AnkA_AR2.T162A, AnkA_AR3.V190A, and AnkA_AR4.M228A using PCR-directed mutagenesis to also created double mutants at ARs 1-2, 2-3 and 3-4. Using fluorescent microscopy co-localization analysis after transfection of HEK293T cells, we found that, with the exception of the region 4 mutation, only mutations of 2 consecutive ARs abrogate AnkA nuclear localization, providing support for the hypothesis. We speculate that the single 4th AR mutation abrogates nuclear transport because ARS: i) is distantly downstream, and/or ii) lacks a hydrophobic residue. By using Importazole, an inhibitor of importin-dependent nuclear transport of proteins, and by loading HEK cells with GTP-γS, a non-hydrolyzable GTP antagonist for RanGDP binding, we found that AnkA protein nuclear transport is importin-independent but potentially occurs through Ran-GDP binding, similar to that established human ankyrin-repeat containing proteins.
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Host, Vector, and Pathogen Interactions I
Pathogenesis/Pathophysiology/Cell Biology II
Host, Vector, Pathogen Interactions II
Infection, Diagnosis & Treatment

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Identification and characterization of a novel adhering receptor for spotted fever group rickettsiae

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Abstract #119

Spotted fever group (SFG) rickettsioses are tick-borne zoonotic diseases of global importance caused by obligatory intracellular bacteria of the genus Rickettsia. Although rickettsial infection is controlled by appropriate broad-spectrum antibiotic therapy, untreated or misdiagnosed SFG rickettsioses are frequently associated with severe morbidity and mortality. Endothelial cells (ECs) are the main mammalian host target cells of SFG rickettsiae. The most prominent pathophysiological effect is increased microvascular permeability, causing vasogenic cerebral edema and non-cardiogenic pulmonary edema with potentially fatal outcomes. The underlying mechanism(s) of rickettsial attachment to and anchoring on the endothelial cell luminal surface remains incompletely determined, including how the bacteria overcome shear stress from blood flow prior to host cell invasion. Here we examined the role of endothelial surface annexin A2 (ANXA2) during rickettsial adherence to human endothelial cells. We demonstrated that endothelial surface ANXA2 contributed to rickettsial attachment to endothelial cells as an adhering receptor. In vivo data from an anatomy-based in vivo quantitative bacterial adhesion analysis system revealed that global depletion of ANXA2 diminished rickettsial adherence to the blood vessel luminal surface. Investigation of a protein-protein interaction at a single-molecule level by atomic force microscopy (AFM) biomechanically characterized endothelial apical surface ANXA2 as a receptor for rickettsial adhesion out membrane protein-B to bind. Coupled with site-directed mutagenesis investigation of protein-living cell interaction with AFM probed phosphorylation of the N-terminus of ANXA as a switch to control rickettsial adhering. Our study, targeting the pivotal initial step in successfully establishing bacterial infection, delineates both biomechanical and biochemical mechanisms underlying rickettsiae hijack endothelial surface ANXA2 for their adherence to non-phagocytic vascular endothelial cells.

Abstract #120

Crossbreeding Amblyomma maculatum group tick populations from distinct geographical regions within the U.S.

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The Amblyomma maculatum group of ixodid ticks currently contains three species as established by Estrada-Peña et al. 2005: A. maculatum, A. triste, and A. tigrinum. However, since Koch described this group in 1844, the systematics of its members has been the subject of ongoing debate. This is especially true of A. maculatum and A. triste; recent molecular analyses reveal insufficient genetic divergence to separate these as distinct species. Further confounding this issue is the unexpected discovery in 2014 of A. maculatum group ticks in southern Arizona, USA that were morphologically identified as A. triste. To biologically resolve the species identity of A. triste from southern Arizona, we evaluated the reproductive compatibility between specimens of A. maculatum collected from Georgia and specimens identified as A. triste that were collected from southern Arizona. Female ticks from the parent generation were mated with males from both the Georgia A. maculatum and Arizona A. triste populations, creating 2 homologous and 2 heterologous cohorts of ticks: GA ♂ /GA♀, GA ♂ /AZ♀, AZ ♂ /AZ♀, and AZ ♂ / GA♀. Each cohort was maintained separately into the F2 generation with F1 females mating only with F1 males from their same cohort. Survival parameters were measured for all developmental stages,
including feeding duration, feeding success, and molting success. In addition, engorgement weight, hatching success, fertility, and fecundity were measured for females of the parent and F1 generations. The observed survival parameters for heterologous cohorts were comparable to those of the homologous cohorts through the F1 generation. Feeding success of the F1 adults was comparable for all cohorts as were both engorgement weight and egg clutch size. Measurement of fertility parameters and hatching success of the F1 generation is ongoing. These data support the recent molecular analyses, to suggest that the populations of A. maculatum group ticks from Arizona and Georgia should be capable of interbreeding.

Abstract #121
Dynamics of R. rickettsii transmission at the tick/host interface
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It has been reported that flat/starving ticks do not transmit spotted fever group Rickettsia immediately upon attachment because pathogenic bacteria exist in a dormant - uninfecious state and require time for “reactivation” before it can be transmitted to a susceptible host. To clarify the length of reactivation period, we exposed guinea pigs to bites of R. rickettsii-infected Dermacentor variabilis and allowed ticks to remain attached for predetermined time periods from 0 to 48 hours. Following removal of attached ticks, salivary glands were immediately tested by PCR, while guinea pigs we observed for 10-11 days post-exposure. Guinea pigs in a control group were subcutaneously inoculated with salivary glands from unfed D. variabilis from the same cohort. In a parallel experiment, skin at the location of tick bite was also excised at the time of tick removal to ascertain dissemination of pathogen from the inoculation site. Animals in every exposure group developed clinical and pathological signs of infection. Even attachments for less than 8 hours resulted in clinically identifiable infection in some guinea pigs. The severity of rickettsial infection in animals increased with the length of tick attachment. Several of the control guinea pigs also developed signs of disseminated rickettsial infection. These findings suggest that the previously reported delay in horizontal transmission of Rickettsia by infected ticks is related to the quantity of the pathogen being delivered by ticks during feeding rather than required “reactivation period”. Results of our study also indicate that R. rickettsii present in unfed ticks does not necessarily require a reactivation period and infected ticks can transmit infectious pathogen virtually as soon as they attach to the host.

Abstract #122
Comparative virulence of diverse Coxiella burnetii strains in vivo
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Coxiella burnetii is the intracellular, gram-negative bacterium that causes the zoonosis Q fever. This disease typically presents as an acute flu-like illness in humans but may result in more persistent, focalized infections, particularly in immunocompromised individuals. Clinical outcomes of Q fever are associated with distinct genomic groups of C. burnetii suggesting that gene content may be responsible for virulence potential. To investigate this hypothesis, the virulence of thirteen C. burnetii isolates was evaluated in a female Hartley guinea pig infection model by intraperitoneal injection (10^5 organisms). These isolates spanned six genomic groups (Groups I-VI). Seven isolates caused a sustained fever (>39.5°; at least two days) in at least 50% of the animals within each experimental group (n=4). Fourteen days post infection animals were euthanized and additional endpoints were evaluated, including bacteremia, body weight index, and splenomegaly. The magnitude of these endpoints roughly correlated with the appearance and severity of fever. The most severe disease was caused by group I isolates. Intermediate and no virulence were evidenced following infection with group II-V and group VI isolates, respectively. Flow cytometric analysis of the mesenteric lymph nodes revealed decreased CD4+ T cell frequency following infection with virulent group I isolates. C. burnetii-specific IgG antibody titers in the sera of infected guinea pigs were variable among all strains, regardless of genomic grouping. These findings suggest unique virulence potentials among distinct Coxiella genomic groups and prompt further investigation into diverse immune responses during infection, particularly at multiple time points post infection. Guinea pigs are considered to be the most physiologically-relevant rodent model of human Q fever and intraperitoneal infection provides an accessible method to determine C.
burnetii virulence. Additionally, the incorporation of novel endpoints allows for more robust analysis of C. burnetii pathogenesis in vivo and identification of immune correlates related to infection clearance.

Abstract #123

Investigating the Role of Metabolic Genes in Intracellular Replication of Coxiella burnetii
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Coxiella burnetii, the causative agent of the neglected zoonotic disease Q fever, is an intracellular Gram-negative bacterium. Although improved genetic tools and the ability to grow the bacterium in host cell-free media has advanced the study of C. burnetii pathogenesis, the mechanisms that allow the bacterium to survive and replicate inside the hostile phagolysosome are not well-understood. Recently we screened a transposon mutant library for replication within HeLa cells and identified a number of genes required for efficient intracellular replication, including two genes with predicted metabolic functions, nadB and cbu_1276, but the actual function of these genes is unknown. To confirm the role of NadB and CBU_1276 in intracellular replication, complementation of the relevant transposon mutants with pJB:Kan plasmid expressing nadB or cbu_1276 with an N-terminal epitope FLAG was carried out. Intracellular replication assays inside HeLa cells using qPCR (quantitative) and fluorescence microscopy (qualitative) were conducted. To systematically analyse differences in the metabolite profiles of the strains, we used an untargeted metabolomics approach to compare the metabolite profiles of wild-type, mutant and complemented strains. Bioinformatics analysis of the NadB amino acid sequence revealed the presence of a conserved arginine residue at position 275 that is usually necessary for catalytic activity. Site-directed mutagenesis was performed to mutate this residue to a leucine, and both wildtype and R275L NadB-GST fusion proteins were expressed in and purified from E. coli JM109. Fluorescent microscopy revealed that 3 days post-infection the nadB and cbu_1276 transposon mutants displayed smaller vacuoles containing fewer bacteria as compared to wildtype and complemented strains. The quantitative intracellular growth assays showed that both the nadB and cbu_1276 transposon mutants also exhibited only a very small increase in genome equivalents (GE) at 7 days post infection in HeLa cells, which was significantly lower than the GE increase for both wild type and the complemented mutants. Liquid chromatography-mass spectrometry analysis also identified significant differences in metabolites related to nicotinamide adenine dinucleotide (NAD), corresponding to the predicted role of NadB in NAD synthesis. Furthermore, enzyme assays using recombinant wildtype NadB-GST demonstrated typical L-aspartate oxidase activity. Future work will include functional characterization of R275L NadB-GST and complementation of the nadB mutant with this mutant protein, as well as characterising CBU-1276 using genetic and biochemical techniques, to investigate and confirm the molecular mechanisms that underlie the role of NadB and CBU-1276 in replication.

Abstract #124

Altering lipid droplet homeostasis affects Coxiella burnetii intracellular growth
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Coxiella burnetii is an obligate intracellular bacterial pathogen and a causative agent of culture-negative endocarditis. While C. burnetii initially infects alveolar macrophages, it has also been found in lipid droplet (LD)-containing foamy macrophages in the cardiac valves of endocarditis patients. In addition, transcriptional studies of C. burnetii-infected macrophages reported differential regulation of the LD coat protein-encoding gene perilipin 2 (plin-2). To further investigate the relationship between LDs and C. burnetii, we compared LD numbers using fluorescence microscopy in mock-infected and C. burnetii-infected alveolar macrophages. On average, C. burnetii-infected macrophages contained twice as many LDs as mock infected macrophages. Increased LD numbers were observed as early as 24 hours post-
infection and was reversed by blocking *C. burnetii* protein synthesis. Further, we observed that the LD accumulation was dependent on the *C. burnetii* Type 4B Secretion System (T4BSS), a major virulence factor that manipulates host cellular processes by secreting bacterial effector proteins into the host cell cytoplasm. To determine the importance of LDs during *C. burnetii* infection, we assessed the effect of manipulating LD homeostasis on *C. burnetii* intracellular growth. Surprisingly, blocking LD formation with the pharmacological inhibitors triacin C or T863, or knocking out acyl-CoA transferase-1 (acat-1) in alveolar macrophages, increased *C. burnetii* growth at least 2-fold. Conversely, preventing LD lipolysis by inhibiting adipose triglyceride lipase (ATGL) with atglistatin almost completely blocked bacterial growth, suggesting LD breakdown is essential for *C. burnetii*. Together these data suggest that maintenance of LD homeostasis, possibly via the *C. burnetii* T4BSS, is critical for bacterial growth. Ongoing experiments are addressing the contribution of LDs as a nutrient source and/or as a site for host cell lipid immune mediator production during *C. burnetii* infection.

**Abstract #125**

**Molecular tuning of tick cell signaling by a rickettsial pathogen**

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Human anaplasmosis, caused by the obligate intracellular rickettsial pathogen *Anaplasma phagophotomilum*, is one of the most common tick-borne diseases in the United States. The black-legged *Ixodes scapularis* ticks are the primary vectors for this bacterium. Upon ingestion, *A. phagophotomilum* enters the tick gut and then colonizes in the salivary glands. These bacteria are not transmitted transovarially but are maintained transstadially through different developmental stages of ticks. Despite numerous studies that have focused in understanding *A. phagophotomilum* interactions with the mammalian host, relatively few studies have focused in understanding survival strategies of *A. phagophotomilum* within ticks. The efforts from my laboratory are focused in the use of both ticks and tick cells to understand dynamics of *A. phagophotomilum*-tick interactions. This study provides evidence on the roles of three arthropod molecules involving organic anion transporting polypeptide (OATP), kynurenine aminotransferase (KAT) and transcriptional activator protein 1 (AP-1) in tick-*A. phagophotomilum* interactions. *A. phagophotomilum* significantly induces expression of these three molecules and RNAi knockdown significantly affects its survival in these ticks. In addition, treatment of tick cells with exogenously added Xanthurenic acid, a product from KAT enzyme, induces OATP4056 expression and increases *A. phagophotomilum* burden. Collectively, this study not only defines molecular mechanism of tripartite tuning by a rickettsial pathogen for its survival in its vector host but also may lead for the development of better strategies to block transmission of *A. phagophotomilum* and perhaps other rickettsial species of medical importance from these ticks.

**Abstract #126**

**Anaplasma phagophotomilum** Asp14 Exploits Host Cell Surface Protein Disulfide Isomerase Activity to Promote Infection

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*Anaplasma phagophotomilum* (Ap) is an obligate intracellular bacterium that invades neutrophils to cause human granulocytic anaplasmosis. How *Ap* invades host cells is poorly detailed. The *Ap* adhesin, Asp14 (14-kDa *Ap* surface protein), contributes to this process. Asp14 facilitates bacterial entry by virtue of its C-terminus, but its receptor has remained undefined. Here, yeast two-hybrid analysis identified host protein disulfide isomerase (PDI) as a putative Asp14 binding partner. Co-immunoprecipitation verified the interaction and determined that it requires the Asp14 C-terminus. PDI knockdown reduced *Ap* infection of, but not binding to host cells. PDI acts solely as a reductase at eukaryotic cell surfaces. *Ap* infection of host cells whose surfaces had been treated with the monoclonal antibody BD34, which catalytically inactivates PDI, was significantly impaired. Bacterial infectivity of BD34-treated cells was restored when *Ap* surfaces but not host cell surfaces were exposed to the reducing agent, tris(2-carboxyethyl)phosphine. Thus, PDI-reduction of an *Ap* but not host cell surface protein is critical for the bacterium’s ability to infect. *Ap* failed to productively infect conditional knockout mice in which myeloid cells lacked PDI. These data suggest that (1) Asp14 interacts with PDI to bring
the bacterial surface in close proximity to enable PDI-mediated reduction of Ap surface disulfides, which, in turn, is key for infection of host cells and (2) the relevance of this mechanism extends in vivo. Given that other intracellular pathogens, including *Chlamydia trachomatis* and HIV, also utilize PDI to invade host cells by incompletely understood means, the details uncovered here could have far reaching implications.

**Abstract #127**

*E. CHAFFEENSIS TRP120 INVOLVED IN ACTIVATION OF CONSERVED SHH PATHWAY*

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The emerging life-threatening zoonosis, human monocytotropic ehrlichiosis (HME), is caused by *Ehrlichia chaffeensis*, an obligately intracellular Gram-negative bacterium that infects mononuclear phagocytes. *E. chaffeensis* TRP120 effector activates conserved host cell signaling pathways like Notch and Wnt to evade host immune defense and promote pathogen internalization and replication. The premise of this investigation is based on our published data that demonstrates that *E. chaffeensis* TRP120 stimulation of THP-1 results in transcriptional upregulation of the Glia-associated oncogene homolog 1(GLU1), a key transcriptional activator of the Sonic Hedgehog (Shh) pathway, which is recently been associated with the regulation of cell survival and apoptosis. The current study is focused on understanding the mechanism of Shh pathway activation during *E. chaffeensis* infection and its role in ehrlichial survival in host. We have demonstrated that *E. chaffeensis* TRP 120 interacts with a host cell surface Shh receptor and promote nuclear translocation of GLU1. Moreover, PCR array analysis of 84 Shh pathway related genes in *E. chaffeensis* infected and uninfected cells demonstrates a significant difference in gene transcription during early (46.4%) and late (35.7%) stages of infection, indicating high pathway activity. Moreover, we have also seen a decreased level of SuFu, a major negative regulator of Shh pathway, suggesting active deregulation of Shh pathway during late stages of infection. These data strongly suggest activation of Shh takes place during *E. chaffeensis* infection in monocytes and involves ehrlichial TRP120 effector and host cell surface Shh receptor, but the complete mechanism of Shh pathway activation and its role in inhibition of host cell apoptosis during infection is not known and is currently under investigation.

**Abstract #128**

*Ehrlichia chaffeensis* Incorporates Host Membrane Components

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The obligatory intracellular small Gram-negative bacterium, *Ehrlichia chaffeensis* needs to acquire a large amount of membrane within parasitophorous vacuoles to multiply. However, there is little information on the trafficking of membrane lipids to membranes of bacteria-containing vacuoles or this intracellular pathogen. Here, we report unidirectional translocation of the lipophilic carboxyamine Dil-prelabeled membrane from host cell plasma membrane to the membranes of parasitophorous vacuole (“inclusion” in which *E. chaffeensis* replicates), intra-inclusion vesicles, and individual *Ehrlichia* organism. Exogenous Bodipy-cholesterol was also incorporated into membranes of ehrlichial infections and the individual bacterium within the inclusion. *E. chaffeensis* replication centers on RAB5-regulated autophagy induced by an ehrlichial type IV secretion effector, Etf-1. Rapamycin stimulation of cellular autophagy enhanced bacterial infection accompanied with increased intra-inclusion Dil and Bodipy-cholesterol-labeled membranes. 3D imaging of *Ehrlichia*-infected cells with FIB-SEM (focused-ion-beam scanning electron microscopy) and immuno-labeling of thin cryo-sections from chemically fixed cells shows numerous, likely host-derived membranous structures in the inclusions. Our studies suggest autophagy supplies host-derived cholesterol-rich intra-inclusion membranes, which serve as the source of glycerophospholipids and cholesterol for proliferation of intravacuolar *E. chaffeensis*. 

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Abstract #129
Outer membrane protein B enables *Rickettsia parkeri* to evade antibacterial autophagy
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Bacterial pathogens in the genus *Rickettsia* strictly grow in the cytosol of host cells where they presumably evade degradation via autophagy by hitherto unknown mechanisms. For other cytosolic bacterial pathogens, bacterial-derived surface molecules are required to evade antibacterial autophagy. We therefore hypothesized that a surface protein protects *Rickettsia* against autophagy. *R. parkeri* transposon mutants lacking surface proteins, including outer membrane protein B (OmpB, also known as rOmpB), were tested for colocalization with autophagy markers. The data showed that OmpB, the major constituent of a proteinaceous surface layer, is a key player in blocking autophagic recognition by polyubiquitylation through acting locally on the bacterial surface. Electron microscopy analysis further showed that OmpB-deficient bacteria lacked an electron-lucent capsular-like halo, indicating that OmpB is a key player for the formation of surface layers that surround *R. parkeri*. In endothelial cells, the growth of OmpB-deficient bacteria was similar to that of wild type bacteria, suggesting that endothelial cells are unable to degrade *R. parkeri* that were subject to autophagic recognition. In contrast, OmpB-deficient bacteria did not grow in wild-type macrophages, but grew in macrophages that genetically lack autophagy components, demonstrating that OmpB is required for autophagy avoidance and bacterial growth in macrophages. To gain further insights into the mechanisms by which OmpB protects *R. parkeri* from autophagic recognition, we used mass spectrometry to identify candidate surface proteins on OmpB-deficient bacteria that were marked with polyubiquitin. These candidates are conserved in Gram-negative bacteria, and hence may represent conserved targets for the host ubiquitin-machinery to initiate antibacterial actions including autophagy. Finally, we showed that OmpB is absolutely required for *R. parkeri* colonization of the organs of C57BL/6 mice. Taken together, these results demonstrated that OmpB is a key virulence factor that enables *R. parkeri* to evade autophagy.

Abstract #130
Distinct ank repeats mediate *Anaplasma phagocytophilum* AnkA-DNA binding and repression of host *CYBB* expression
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*Anaplasma phagocytophilum*, which invades human neutrophils, is an obligate intracellular pathogen. *A. phagocytophilum* produces the protein AnkA which utilizes type 4 secretion to enter the host cell. AnkA then binds DNA and silences host defense genes, including *CYBB*. This action dampens neutrophil respiratory burst. We previously demonstrated that binding of AnkA at the *CYBB* promoter leads to HDAC1 recruitment and POL II exclusion. AnkA has 15 ankyrin repeats (AR) that are distributed throughout the N-terminus and the central part of the protein. C- and N-truncation of AnkA that includes the central 8 ARs diminishes both DNA binding and promoter silencing activity. To better localize and discriminate the role of ARs in these functions, we deleted consecutive ARs spanning repeats 5-15. Constructs deficient in adjacent ARs were examined for DNA binding of mutants by EMSA using a *CYBB* promoter probe. DNA silencing activity was studied using HEK 293T cells transfected with a *CYBB*/β-galactosidase reporter and the AR deletion mutants. Transfection of AnkA that lacks ARs 5-8, 7-10 and 9-12 did not alter binding to labeled *CYBB* reporter, but transfection with AnkA lacking ARs 11-15 and 14-15 bound to the *CYBB* reporter less than wildtype AnkA. All AR deletion mutants had impaired silencing activity compared to wildtype AnkA (p<0.013-0.0003) but this was most pronounced in the AR 7-10 (p=0.0005) and 9-12 deletion mutants (p=0.0003). From these studies, we show that transcriptional repression and DNA binding can be attributed to distinct ARs in AnkA. The DNA binding property of AnkA is attributed largely to ARs 11-15 and silencing activity to ARs 7-12. Mutant AnkAs dissociated of these functions will provide important tools to dissect precise mechanisms by which AnkA functions in gene silencing and reconfiguration of nuclear architecture that are suspected to contribute to host cell transcriptional reprogramming.
Abstract #131
Identification of a Notch activation motif in Ehrlichia chaffeensis TRP120 effector
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Human monocytotropic ehrlichiosis is an emerging, life-threatening tick-borne infectious disease caused by Ehrlichia chaffeensis, an obligately intracellular bacterial pathogen. Recent studies by our laboratory have shown that the TRP120 effector is able to activate the Notch pathway during infection to downregulate innate pattern recognition and potentially apoptosis. We have demonstrated that TRP120 directly interacts with ADAM17, a Notch metalloprotease, and colocalizes with both ADAM17 and the Notch receptor. Recent studies have demonstrated inhibition of host cell apoptosis as a mechanism utilized by E. chaffeensis for survival. Notch activation has shown to assist in inhibition of apoptosis by stabilizing expression of an anti-apoptotic protein, X-Linked Inhibitor of Apoptosis (XIAP). Although we have elucidated a novel pathogen-host interaction that exploits Notch signaling as a mechanism to evade host innate immune response to enhance infection, the nature of the molecular interactions between E. chaffeensis TRP120 and the Notch receptor complex are relatively unknown. The purpose of this study is to identify the molecular and functional interactions and mechanisms responsible for TRP120 Notch activation. Therefore, we hypothesize that E. chaffeensis TRP120 is a novel non-canonical Notch ligand that activates Notch signaling via tandem repeat amino acids molecularly interacting with Notch receptor EGF domains, and ADAM17 membrane proximal domains, to inhibit apoptosis via upregulation of XIAP. Using sequence homology databases, immunofluorescence microscopy and WB analysis, we have identified sequence homology between TRP120 and several non-canonical Notch ligands. Specifically, a four amino acid motif in the tandem repeat domain of TRP120 has been shown to be important for Notch activation. We further demonstrated an increase in XIAP expression at later time points of infection. Taken together, these results indicate that a specific TRP120-TR sequence is important for the activation of Notch, and an increase in XIAP expression levels is occurring following Notch activation during E. chaffeensis infection. Further analysis of TRP120-Notch-R1/ADAM17 interaction will be studied using biochemical based assays and crystallography.

Abstract #132
Distinct bacterial stages during the intracellular infection cycle of Orientia tsutsugamushi
Suparat Giengkam, Jantana Wongsantichon, Thomas Pouplin, Graham Wright and Jeanne Salje*

It is well established that some bacterial cells can differentiate into distinct and stable cell types. This differentiation can be developmentally programmed (such as the stalked/swarmer cells of C. crescentus), can arise stochastically (such as persister cells in E. coli) or can arise in response to specific environmental cues (such as differentiation within biofilms of B. subtilis). Developmental differentiation is well described in Anaplasmataceae bacteria, which typically switch between infectious elementary bodies and replicative reticulate bodies. Differentiation in the Rickettsiaceae is less well described, and this is partly because there are few gross morphological differences between bacterial cells at different stages of their infection cycle. In the current study, we set out to explore the distinct developmental stages of the Rickettsiaceae Orientia tsutsugamushi. We used a combination of immunofluorescence microscopy, RTqPCR gene expression analysis, Western blot and proteomics analysis to study differences in the expression and localisation of key bacterial marker proteins at different stages of infection. We used structured illumination microscopy and confocal microscopy to study changes in bacterial morphology over time. We used lipidomics profiling to study differences in lipid composition, and click-chemistry based metabolic labelling in order to determine the timing of initiation of protein synthesis in bacterial cells following entry into host cells. Taken together, we found that Orientia tsutsugamushi exhibits clear and reproducible differences in gene expression, cellular morphology, metabolic activity and lipid composition at different stages after infection. We propose that these differences reflect distinct stages in the intracellular infection cycle of Orientia tsutsugamushi, and this raises further questions about the environmental cues triggering bacterial differentiation, and the biological differences between the different intracellular forms of Orientia tsutsugamushi.
Abstract #133

Rickettsia exploit the inflammasome to avoid the killing effects of type I interferon
Thomas P. Burke*, Patrik Engström, Roberto Chavez, Russell E. Vance, Matthew D. Welch
Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720

The Rickettsia are a diverse group of obligate cytosolic bacterial pathogens that cause severe human disease including typhus and spotted fever; however, it remains poorly understood how Rickettsia interact with the innate immune system. We sought to characterize the host factors governing intracellular growth of the spotted fever group pathogen R. parkeri. The bacteria grew robustly in wild-type macrophages, inducing low amounts of host cell death and low secretion of type I interferon (IFN-I). Curiously, the bacteria were strikingly killed in macrophages lacking components of the inflammasome, which detects cytosolic bacterial ligands to elicit rapid host cell death. Infected inflammasome-deficient macrophages had increased secretion of IFN-I, which was responsible for killing R. parkeri. We then asked what host factors were required for killing R. parkeri downstream of IFN-I and observed that macrophages lacking the transcription factor IRF5 were deficient for bacterial killing. IRF5 was required for the expression of antimicrobial genes including the guanylate binding proteins (GBPs), which were involved with restricting Rickettsia growth after IFN-I treatment. To test this in vivo, we analyzed bacterial colonization of organs in infected C57BL/6 mice. After infection, spleens of inflammasome-deficient mice had increased induction of IFN-I but similar burdens of R. parkeri compared to wild-type mice, whereas mice lacking both the inflammasome and IFN-I signaling pathways had increased bacterial burdens. Interestingly, treatment of infected mice with recombinant IFN-I reduced bacterial burdens. Together, these findings suggest that, unlike facultative pathogens that have evolved sophisticated mechanisms to avoid the inflammasome, Rickettsia tolerates some inflammasome activation to antagonize the killing effects of IFN-I.

Abstract #134

New Insights into the Pathogenesis of Severe Scrub Typhus
Brandon Trent¹, Yan Xing², Yuejin Liang³, Jinjun Liu², Donald Bouyer³, Sahni Sanjeev¹, Jiyang Cai³, Lynn Soong¹,²*
Departments of Pathology¹, Microbiology and Immunology², and Ophthalmology³, Institute of Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX

We have recently established lethal and sublethal Orientia tsutsugamushi infection models in C57BL/6 mice, which mimic clinical/pathologic features of human scrub typhus and are valuable for future mechanistic studies. A hallmark of lethal Orientia infection is Th1-skewed, but Th2-impared, immune responses, accompanied with severe endothelial damage and multi-organ failure, especially in the lungs. Importantly, while tissue bacteria reach peaks at day 6 (the onset of disease) in mice, neutrophil and CD8 T cell influx/activation reach peaks in the lungs at day 10 (the severe disease stage prior to host death). We hypothesize a 2-stage-dysfunction loop for severe scrub typhus: 1) Orientia replication in phagocytes and endothelial cells (EC) triggers inflammatory responses and host defense machinery; 2) infection-mediated release of damage-associated molecular patterns (DAMPs) exacerbates vascular dysfunction, even after the control of bacterial replication. This hypothesis was supported at the tissue level by immunofluorescent co-staining of bacteria with leukocyte-, EC-, and platelet-specific markers, Western blot, and qRT-PCR. Form days 0, 2, 6, and 10 of infection, the progressive loss of vasculature and tight junctions (occludin, VE-cad, lectin positivity) and vascular function (reduced angiopoietin 1, Tie2, and pTie2 levels), as well as reduced CD41+ platelets in the lungs, were positively linked to the frequency/number/activation of neutrophils and CD8 T cells, rather than body bacterial loads. The mechanisms of immune dysregulation were further examined by using infected human endothelial cell cultures and mouse bone marrow-derived macrophages and neutrophils. While Orientia infection can directly alter the Ang-Tie2 axis in EC, IL-33/IL-36-like DAMPs (released from/processed by leukocytes) can contribute to vascular damage and tissue damage. Our studies provide new insights into immune dysregulation and pathogenesis of severe scrub typhus. A better understanding of infection-versus immune-mediated dysregulation and prognostic biomarkers will help the control of this neglected tropical disease.
Abstract #135
Transkingdom Intercommunication at the Vector-Pathogen-Host Interface
Adela S. Oliva Chavez1, Xiaowei Wang1, Holly L. Hammond1, Dana K. Shaw1, Erin E. McClure1, Amanda Buskirk2, Marcela F. Pasetti3, Steven M. Jay3, Kateryna Morozova4, Laura Santambrogio6, and Joao H.F. Pedra11
1Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA; 2Center for Vaccine Development, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA; 3Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA; 4Departments of Pathology and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Approximately 17% of all infectious diseases with public health relevance are arthropod-borne. Ticks, mosquitoes, biting flies and fleas evolved salivary proteins with anti-hemostatic, anesthetic, and anti-inflammatory properties. These molecules counteract mammalian host defenses and facilitate pathogen transmission to humans. Therefore, uncovering how these salivary proteins are transported between the arthropod vector and the mammalian host would leverage the development of unique biomarkers and/or vaccine targets against vector-borne illnesses. Here we demonstrate that CD63, ALIX and TSG101 extracellular vesicles from the tick Ixodes scapularis carry anti-inflammatory effector proteins. Tick extracellular vesicles interact with mammalian immune cells and facilitate transkingdom intercommunication. Two members of the vesicle-associated membrane protein family (VAMPA/VAMP33 and VAMP2/Synaptobrevin 2) affect vesicle biogenesis and tick feeding during a bloodmeal. Manipulation of VAMPA/VAMP33 and VAMP2/Synaptobrevin 2 expression in I. scapularis led to a switch in Th1/Th2 inflammatory biomarkers and alteration of CXCR3, CCR2 and CCR6 chemokine receptor signaling. Intracellular, but not extracellular bacteria, also enhanced tick vesicle secretion and altered carbonyl modifications within its cargo to exploit the disruption of immune homeostasis at the skin site. Collectively, we uncovered a novel paradigm in infectious diseases by establishing extracellular vesicles as a vehicle of contextual pathogenicity in arthropod-borne diseases.

Abstract #136
Dissemination of Orientia tsutsugamushi and Immunological Responses in the Humanized DRAGA Mouse
Le Jiang1, Erin K Morris2, Rodrigo Aguijera-Olvera3, Zhiwen Zhang1, Teik-Chye Chan1, Soumya Shashikumar3, Chien-Chung Chao1,4, Sofia A Casares3,4 and Wei-Mei Ching*1,4
1Viral and Rickettsial Diseases Department, Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD; 2Veterinary Services Program, Department of Pathology Services, Walter Reed Army Institute of Research, Silver Spring, MD; 3US Military Malaria Vaccine Program, Naval Medical Research Center/Walter Reed Army Institute of Research, Silver Spring, MD; 4Uniformed Services University of the Health Sciences, Bethesda, MD

Scrub typhus is caused by Orientia tsutsugamushi, an obligate intracellular bacterium that affects over a million people every year. Numerous mouse models have been used to study its pathogenesis, disease immunology, and for testing vaccine candidates. However, due to the intrinsic differences between the immune systems in mouse and human, these mouse models could not faithfully mimic the pathology and immunological responses developed in human patients, limiting their value in both basic and translational studies. In the present study, we have established for the first time, a humanized mouse model through footpad inoculation of O. tsutsugamushi in DRAGA (HLA-A2.HLA-DR4.Rag1KO.II2RγcKO.NOD) mice with their immune systems reconstituted by infusion of HLA-matched human hematopoietic stem cells from umbilical cord blood. Upon infection, Orientia disseminated into various organs of DRAGA mice and resulted in lethality in a dose-dependent manner. By contrast, conventional C3H/HeJ mice infected by the same route all survived. Tissue-specific lesions associated with inflammation and/or necrosis were observed in multiple organs of infected DRAGA mice. Consistent with the intracellular nature of Orientia, strong Th1, but subdued Th2 responses were elicited as reflected by the human cytokine profiles in sera from these infected mice. Interestingly, the percentage of both activated and regulatory (CD4*FOXP3*) human T cells were elevated in spleen tissues of infected mice. Both humoral and cellular immune responses of human origin were detected after immunization with irradiated whole cell Orientia. These include significant activation of human T cells as evidenced by increased numbers of human CD4* and CD8* T cells and development of specific human IgM/IgG antibodies. This new humanized DRAGA mouse model has major advantages
over previous conventional mouse models and has a great potential to be used for studying pathogen-host interactions and for evaluating vaccine candidates or other therapeutics for scrub typhus.

Abstract #137
A pathogen and a non-pathogen SFG *Rickettsia* trigger differential metabolic signatures in macrophage-like cells
Pedro Curto1,2,3,4, Cátia Santa3, Bruno Manadas3, Isaura Simões3,4*, Juan J. Martinez4
1PhD Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal; 2Institute for Interdisciplinary Research, University of Coimbra, Portugal; 3CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal; 4Vector Borne Disease Laboratories, Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA, United States

Reductive genome evolution in obligate intracellular *Rickettsia* has resulted in the loss of many metabolic pathways, which culminates with *Rickettsia* species being strictly dependent on host cells to survive and proliferate. Several efforts have been made to identify host and bacterial determinants that allow bacteria to proliferate inside host cells. We recently reported a differential tropism of pathogenic and non-pathogenic *Rickettsia* in macrophage-like cells, further strengthening the complexity of host-rickettsiae interactions and raising questions on how pathogenic *Rickettsia* manipulate host pathways to their advantage. To further understand this, we have herein employed a quantitative high-throughput proteomics approach (SWATH-MS) to profile alterations in THP-1 macrophages infected with *R. conorii* and *R. montanensis*. Interestingly, our results revealed that *R. conorii* is able to substantially reprogram several host metabolic pathways, modulating host cells to a niche apparently more adapted to its metabolic needs. Although both species triggered a decrease in the abundance of several glycolytic and pentose phosphate pathway enzymes, infection with *R. conorii* specifically resulted in an overrepresentation of host TCA cycle enzymes, suggesting that glutamate may be the major anaplerotic substrate. Moreover, infection of macrophage-like cells with *R. conorii* augmented the abundance of host enzymes involved in lipid catabolism and anabolism, suggesting that lipophagy may play an important role in rickettsial intracellular lifestyle. Overall, our proteomic profiling of rickettsiae-macrophage interaction reveals a profound metabolic rewriting of host cells by *R. conorii* that may allow bacteria to obtain the building blocks necessary for replication and, simultaneously, assure the energetic demands of the host cell. By providing new insights on the effect of *R. conorii* on macrophage metabolism, this work adds further knowledge on pathogenicity requirements in rickettsiae and, as excitingly, may help to decipher the still missing ingredients for the design of an axenic culture medium for *Rickettsia*.

Abstract #138
Dispersal of the trans-Golgi network by the effector RARP2 of *Rickettsia rickettsii* inhibits host cell protein transport to the cell surface
Karin Aistleitner*, Tina Clark and Ted Hackstadt
Rocky Mountain Laboratories, NIH, Hamilton, MT

The trans-Golgi network is the major secretory pathway sorting station that directs newly synthesized proteins to different subcellular destinations. We show here that virulent strains of the obligate intracellular bacterium *R. rickettsii* cause dispersal of the trans Golgi, but not the cis Golgi network in host cells. The trans-Golgi is dispersed shortly after entry of *R. rickettsii* into host cells and stays dispersed throughout the whole infection process. While dispersal occurs in both Vero and human dermal microvascular endothelial cells infected with virulent *R. rickettsii* strains, the Golgi is not affected in tick cells which represent the reservoir of *R. rickettsii* in nature. The dispersal is caused by the secretion of the rickettsial Ankyrin repeat protein 2 (RARP2), a recently identified type IV secreted effector protein. RARP2 is one of the few genes showing differences in genomic comparisons between *R. rickettsii* strains differing in virulence, being truncated in the avirulent Iowa strain compared to virulent strains. The dispersal also interferes with both functions of the Golgi: glycosylation of host cell proteins is incomplete as shown by weight shifts in Western blot analysis and dispersal of lectin staining in infected host cells. In addition, protein transport to the cell surface is severely impaired in cells infected with virulent strains of *R. rickettsii*. This includes trafficking of MHC I and might thereby help virulent strains of *R. rickettsii* to evade the host immune system.
Abstract #139
P47 Restricts Anaplasma phagocytophilum Acquisition by Ixodes scapularis Ticks
Erin E. McClure*, Xiaowei Wang, and Joao H.F. Pedra
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The Ixodes scapularis tick transmits up to seven different pathogens to humans, including the Lyme disease spirochete Borrelia burgdorferi and the rickettsial pathogen Anaplasma phagocytophilum. Despite the public health burden of the I. scapularis tick, detailed knowledge of vector-pathogen interactions is lacking. One interface between the tick vector and A. phagocytophilum is the tick immune system. The immune deficiency (IMD) pathway leads to nuclear factor (NF)-κB-mediated production of antimicrobial peptides upon stimulation. The central regulator of the IMD pathway in ticks is the E3 ubiquitin ligase X-linked inhibitor of apoptosis (XIAP). We hypothesized that substrates of XIAP regulate the IMD pathway in I. scapularis. Coimmunoprecipitation of XIAP and associated proteins followed by mass spectrometry led to the identification of p47, a highly conserved protein canonically involved in regulating membrane biogenesis. Silencing of p47 in I. scapularis nymphs led to increased A. phagocytophilum acquisition. We showed that XIAP interacts with p47 and ubiquitylates p47 in a K63-dependent manner. We also demonstrated that p47 is required for IMD pathway activation upon A. phagocytophilum infection. Together, these results indicate that p47 may interact with XIAP to function as a positive regulator of the IMD pathway in ticks. Future directions include elucidating the mechanism of p47-mediated inhibition of A. phagocytophilum acquisition by I. scapularis.

Abstract #140
Phenotypic characterization and comparison among geographically different isolates of Rickettsia rickettsii
Maria F. B. M. Galletti1, Joy A. Hecht1, Jana M. Ritter2, Brad J. Biggerstaff3, Christopher D. Paddock1, Sandor E. Karpathy1
1Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA;
2Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA;
3Division of Vector-Borne Diseases, Office of the Director, Centers for Disease Control and Prevention, Atlanta, GA

Rickettsia rickettsii is the etiologic agent of life-threatening and rapidly progressing tick-borne disease, Rocky Mountain spotted fever (RMSF). Previous studies identified genetic differences among various strains of R. rickettsii that correlate directly with their geographical origin. Interestingly, differences in case fatality rates of RMSF are also reported among distinct geographical regions. In this work, using a Cavia porcellus model, we explore the differences among 6 geographically different R. rickettsii isolates from the American continent (Iowa, Sheila Smith, Sawtooth, Gila, Costa Rica, and Taiauc). All animals were intraperitoneally injected with an equal number of viable rickettsiae and infection in each animal was monitored daily by measuring weight and rectal temperature. At 6 days post infection, animals were necropsied for collection of 5 different tissues in addition to blood. Gross pathology and histopathology were scored. Three different phenotypes were identified based on our animal model data (avirulent, mildly virulent and highly virulent groups) and histological and immunohistochemical analysis corroborated the progressive inflammatory responses associated with these 3 phenotypes. In addition, rickettsial load analysis (qPCR) in all individual samples suggest a strain-tissue preference at this time of infection. Collectively, these results highlight the contribution of the rickettsial strain to the pathogenic response of the host. Phenotypical characterization and comparison among strains from different geographical areas is a necessary step in explaining range of clinical outcomes associated with RMSF.

Abstract #141
Australian SFG rickettsiae in ticks from Queensland, north-eastern Australia.
Haz Hussain-Yusuf1, Amy Shima2, Noel Preece2, Gemma Vincent1, Mythili Tadepalli1, Sze Fui1, John Stenos1 and Stephen Graves1
1Australian Rickettsial Reference Laboratory, Geelong, Victoria; 2James Cook University, Queensland, Australia.

An opportunistic collection of 203 ticks from Queensland, in north-east Australia, contained 9 tick species (mainly Ixodes holocyclus, I.tasmani and Rhipicephalus sanguineus). They were collected from 10 mammalian species; 6 marsupial (mainly tree kangaroo, bandicoot and bettong) and 4 placental (mainly humans and dogs). Overall 13 ticks (6%),
(representing 4 tick species), contained SFG rickettsial DNA and were collected from 6 different mammalian species. *I. tasmani* (29% positive) and *I. holocyclus* (2% positive) were the main tick species containing SFG rickettsial DNA. Three species of rickettsiae were confirmed by sequencing 1 of at least 4 genes (*gltA, 17kDa, Sca 4, Omp B*). These were *Rickettsia gravesii*, Candidatus *Rickettsia antichini* & Candidatus *Rickettsia tasmanensis*. These 3 rickettsiae appear to be widespread in Australia and may be unique to this island continent.

**Abstract #142**

Q fever – A continued threat to the military. Can antibiotic prophylaxis reduce the severity of disease?

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Academic Department, Royal Centre for Defence Medicine (Academia and Research), Birmingham, United Kingdomb; Defence Science and Technology Laboratory, Porton Down, Salisbury, United Kingdomb; Public Health England, Porton Down, Salisbury, United Kingdom; College of Life and Environmental Sciences, University of Exeter, Exeter, UK.

Q fever is caused by the intracellular bacterium *Coxiella burnetii*. It is found worldwide with the exception of New Zealand. It is a zoonotic infection with bacteria concentrated in the birth products of ruminant animals. Infection is largely transmitted via the aerosol route. It was initially identified as a military problem when thousands were affected during WWI. More recently Q fever has been recognised as a problem in UK troops returning from Afghanistan. Approximately 20% of patients develop Q fever fatigue syndrome which can lead to medical discharge. Doxycycline is the first line treatment with quinolones being an alternative. *C. burnetii* is classified as a CDC category B agent. There is no licenced vaccine in the UK against Q fever therefore antibiotic prophylaxis should be evaluated. In an initial study, *A/J* mice were challenged with *C. burnetii* via the aerosol route and treated with 7 days of a range of antibiotics starting either 1 day pre or 1 day post exposure. Weight loss, clinical signs, organ weight at necropsy and bacterial burden of the organs were measured. Doxycycline hyclate and levofloxacin pre and post exposure significantly protected against weight loss compared with the controls (*p*<0.05). Ciprofloxacin and co-trimoxazole provided no significant protection. A follow up study was performed in *A/J* mice focusing on an alternative preparation of doxycycline (doxycycline monohydrate). Therapy was given for either 7 or 14 days, and treatment was commenced either 1 day pre or 5 days post exposure to *C. burnetii*. The delay in post exposure therapy coincides with symptom onset in *A/J* mice. Both pre exposure treatment groups were significantly protected from weight loss compared to the control group (*p* < 0.01), whilst weight loss was significantly reduced in both post exposure treatment groups from 24 hours after therapy was commenced (*p* < 0.05). © Crown copyright (2018), Dstl. This material is licensed under the terms of the Open Government Licence

**Abstract #143**

Development of a serologic tool for differential diagnosis of rickettsiosis

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^[1^]Center for Regional Research “Dr Hideyo Noguchi”, UADY. México;[^2^]Faculty of Medicine, UADY. México

Rickettsiosis is a highly prevalent disease in Mexico, whose differential and timely diagnosis is difficult and challenging as there are other vector borne diseases with similar characteristics, like dengue and Chikungunya. An approach to solve this problem is the use of reverse informatics coupled to recombinant technology to obtain interesting candidates for diagnosis. In this work, recombinant peptides of *Rickettsia sp* (RKT), *Dengue* (DEN) and *Chikungunya* (CHK) viruses were cloned, expressed and purified as recombinant proteins fused to a Hisx6 tag. The utility of these proteins for diagnosis was evaluated by dot-blot and latex agglutination; and compared against the gold standard test for each disease using serum samples from patients coursing different stages of each disease. Sensitivity and specificity values over 90% and 95% respectively were obtained using the dot-blot assay with RKT for IgM and IgG antibodies; over 80% and 90% respectively for DEN; 70% and 80% with CHK. Additionally, there is no cross reactivity among RKT with DEN or CHK however, we observed cross reactivity among DEN and CHK. Same values were analyzed with the latex agglutination tests, obtaining 85% and 100% of sensitivity and specificity respectively using IgG antibodies against RKT; 73% and 98% for DEN respectively; 80% and 91% for CHK. Cross reactivity was similar to that observed with the dot blot. Considering the values observed on the performance, ease and cost of these tests against gold standards, we conclude that these candidates could be useful in a differential and timely diagnosis kit for dengue, chikungunya and rickettsiosis, in order to reduce the
epidemiologic burden in vulnerable regions. Particularly for rickettsiosis, the test seems to be a reliable tool ready for test with a higher sample of patients.

**Abstract #144**

Intervention against tick-borne diseases in a Mayan community
Karla Dzul1*, Gaspar Peniche2, Cesar Lugo,1 Raúl Tello1 and Juan Arias2

1Laboratorio de Enfermedades Emergentes y Reemergentes del Centro de Investigaciones Regionales Dr. Hideyo Noguchi; 2 Unidad Interinstitucional de Investigación Clínica y Epidemiológica

Ticks are the second most important vectors not only because its worldwide distribution but also for the severe diseases that can transmit like rickettsiosis. People living in neglected communities, have a closer contact with ticks due to its activities like the agriculture, cattle-trade, hunting; or its living conditions. Intervention campaigns against vector borne diseases should consider the people knowledge and habits in the strategies aimed to reduce their exposition to those vectors. The objective of this work was to develop a strategy that could be useful for the social intervention of Mayan communities from Yucatan, which considering their social and ecological characteristics of risk to acquire tick borne diseases. This work has been developed in two phases. In first phase we collected and analyzed the social determinants to design intervention campaigns against tick-borne diseases. The second phase consisted in the social intervention of children through talks, social cartography and ludic activities; and adults by workshops and community participation collects. This study showed that older associated factors correspond to the age and poor structural construction of households, unhygienic conditions and anthropogenic environmental practices. In children results it reveals that it is essential to make an educational intervention in this population, schoolchildren are familiar with the vector, but do not know the implication of the role in the transmission of the disease. Both interventions led to a strong empowerment of the knowledge by these people, which could have an impact in the reduction of tick-borne diseases. It is important for Public Health to develop preventive intervention programs in communities of risk.

**Abstract #145**

Novel adjuvant systems elicit unique and protective cellular and humoral immune responses in Q fever challenge model using a polyvalent *C. burnetii* vaccine
Adrienne P. Gilkes*1, Tyler J. Albin1, Saikat Manna2, Medalyn Supnet1, Aarti Jain1, David H. Davies1, Rie Nakajima1, Jiin Felgner1, Sara Ruiz1, Aysegul Nalca3, Aaron Esser-Kahn1, Philip L. Felgner1, Amanda M. Burkhardt1

1 University of California, Irvine, Irvine, CA 92697, USA; 2 The University of Chicago, Chicago, IL 60637, USA; 3 U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702, USA

*Coxiella burnetii* is the causative agent of Q-fever. This disease manifests with flu-like symptoms, but can progress into a fatal chronic infection resulting in endocarditis or neurological manifestations up to 20 years following the initial infection. *C. burnetii* is a Category B bioterrorism pathogen due to its low infectious dose and hearty nature. Despite these facts, the sole vaccine available is only licensed in Australia due to significant safety concerns, which necessitates the development of a less immunogenic yet efficacious vaccine. Previous unsuccessful attempts to develop a protective Q-fever vaccine have identified single immunogenic antigens. Our research group has developed unique, polyvalent anti-*C. burnetii* vaccine candidates that take advantage of two distinct technologies: 1) a protein microarray to identify seroreactive *C. burnetii*proteins and 2) novel adjuvanting systems. We have used the protein microarray to identify several highly immunogenic *C. burnetii* proteins from sera of previously infected humans and combined these with our novel adjuvants, which include polymers and conjugated Toll-like receptor agonists. We have previously shown that our adjuvants elicit antigen specific B and T cell responses and successfully increase antibody scope and diversity compared to non-adjuvanted vaccine candidates, suggesting downstream changes in immune signaling and adaptive immune activation. We have tested these *C. burnetii* vaccine candidates *in vitro* and *in vivo* for safety, immunogenicity and ability to protect animals in a *C. burnetii* aerosol challenge. Our studies suggest that our novel polyvalent *C. burnetii* vaccine could be an effective and safer alternative to Q-Vax®.
## ASR 2018 Travel Award Recipients

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