



American
Society for
Rickettsiology



30th Meeting

Santa Fe, NM
June 8-11, 2019

**Rickettsial Diseases
at the
Vector-Pathogen Interface**

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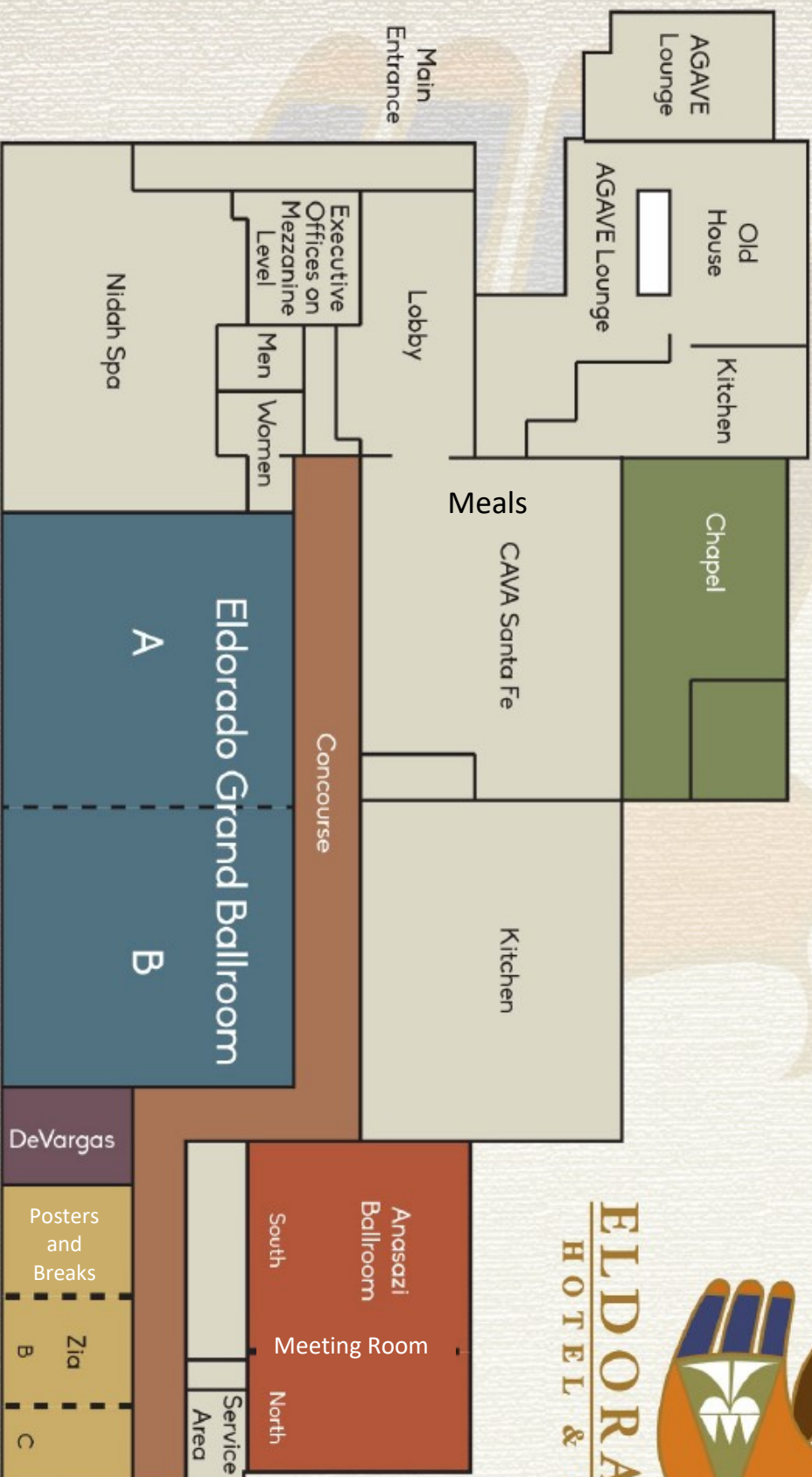
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Funding for this conference was made possible [in part] by R13 AI126727-01 from the **National Institute of Allergy and Infectious Diseases**. The views expressed in written conference materials or publications and by speakers and moderators do not 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.



ELDORADO HOTEL & SPA



American Society for Rickettsiology: Rickettsial Diseases at the Vector-Pathogen Interface



**June 8-11,
2019
El Dorado Hotel,
Santa Fe, New Mexico**



Oral presentations will be held in the
Anasazi Ballroom

Poster presentations will be held in the
Zia Ballroom

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Schedule at a Glance

Saturday June 8	Sunday June 9	Monday June 10	Tuesday June 11
All talks/Sessions and Business Meeting will be in the Anasazi Ballroom	Breakfast in the Cava Lounge 7:00-8:00		
	NIH funded Workshop	Session 5: Eco/Epi 8:00-10:05	Session 8: T4 Effectors 8:00-9:50
	Session 1: Ticks 8:00-9:40	Break	Break
	Break	Session 6: Vacc/Inf, Diag & Treat 10:30-11:45	Session 9: Omics 10:30-12:00
	Session 2: Fleas and Lice 10:10-12:20	Free Afternoon Optional Outing to Taos	Lunch and Zia Posters
	Lunch on your own		
Registration noon->	Session 3: Microbiome 2:00-3:45		Session 10: Path I 2:00-3:15
	Posters Zia Ballroom 3:45-5:30	Dinner on your own	Break
	Special Symposium 3:00-6:00		Session 11: Path II 3:45-5:00
	Historical Lecture 6:30-7:30		Business Meeting 5:00-6:00
Mixer Presidential Suite 7:30-9:30	Session 4: Mentoring Session 7:30-9:30	Session 7: H-P-V Interactions 7:30-9:30	Gala Dinner Cava Lounge 7:00-9:00

SATURDAY

Special Symposium

Chair: Janet Foley & Chris Paddock

Time: 15:00 - 18:00

Date: 8th June 2019

Location: Anzasazi Ballroom

62 -

Missing elements of natural history and ecology in Rickettsiology

Janet Foley

University of California, Davis, USA

72 -

Rocky Mountain Spotted Fever and North Asian Tick Typhus: two diseases, the history, geography, diversity of the tick vectors, and common problems in the modern world.

Marina Ereemeeva

Georgia Southern University, Statesboro, USA

65 -

What we know and what we don't know about the ecology of *Rhipicephalus sanguineus* transmitted rickettsias in the Mediterranean area

Philippe Parola

IHU Méditerranée Infection, Marseille, France

155 -

The need for integrative approaches to deal with Rocky Mountain spotted fever in Sonora, Mexico

Gerardo Alvarez-Hernandez

University of Sonora, Hermosillo, Mexico

96 -

Deciphering the ecology of flea-borne spotted fever

Kevin Macaluso

Louisiana State University, Baton Rouge, USA

151 -

The Ecology of Murine Typhus in the United States

Lucas Blanton

University of Texas Medical Branch, Galveston, USA

149 -

Ecology of *Amblyomma maculatum* and *Rickettsia parkeri*

Holly Gaff

Old Dominion University, Norfolk, USA

56 -

Changing patterns and perceptions of tick-borne rickettsioses in western North America

Christopher Paddock

Centers for Disease Control and Prevention, Atlanta, USA

Historical Lecture: Sir Arnold Theiler

Chair: Kelly Brayton

Time: 18:30 - 19:30

Date: 8th June 2019

Location: Anzasazi Ballroom

Kathy Kocan and Ed Blouin

Mixer and Cocktails

Time: 19:30 - 21:30

Date: 8th June 2019

Location: Presidential Patio

SUNDAY

Breakfast

Time: 7:00 - 8:00

Date: 9th June 2019

Location: Cava

Session 1: Ticks

Chair: Ulrike Munderloh & Susan Noh

Time: 8:00 - 9:40

Date: 9th June 2019

Location: Anzasazi Ballroom

153 -

Tick Biology

Lorenza Beati

Georgia Southern University, Statesboro, USA

85 -

Under Pressure: Stress Response at the Pathogen-Vector Interface

Kristin Rosche, Lindsay Sidak-Loftis, Shannon Allen, Brittany O'Keeffe, Dana Shaw

Veterinary Microbiology and Pathology, Washington State University, Pullman, USA

126 -

Tick Immunity: Understanding the *Rickettsia parkeri* infection in *Amblyomma maculatum* tick interactions through innate immunity and redox signaling pathways.

Faizan Tahir

University of Southern Mississippi, Hattiesburg, USA

135 -

Wiring tick signaling circuitry by a rickettsial pathogen

Girish Neelakanta

Center for Molecular Medicine, Department of Biological Sciences, Old Dominion University, Norfolk, USA

66 -

Different features of *Amblyomma sculptum* and *Amblyomma aureolatum* midgut delineate their distinct susceptibility to infection with *Rickettsia rickettsii*

Nicolas Schröder¹, Flávia Ferreira², Larissa Martins², Maria Galletti^{2,3}, Daniel Pavanelo², José Ribeiro⁴, Marisa Farber⁵, Marcelo Labruna², Sirlei Daffre², Andrea Fogaca²

¹Institute of Biomedical Sciences/University of São Paulo, São Paulo, Brazil. ²University of São Paulo, São Paulo, Brazil.

³Centers for Disease Control and Prevention, Atlanta, USA. ⁴National Institute of Allergy and Infectious Diseases,

Bethesda, USA. ⁵National Agricultural Technology Institute, Buenos Aires, Argentina

Break

Time: 9:40 - 10:10

Date: 9th June 2019

Location: Zia Ballroom

Coffee Break

Session 2: Fleas and Lice

Chair: Alison Fedrow & Mike Minnick

Time: 10:10 - 12:20

Date: 9th June 2019

Location: Anzasazi Ballroom

140 -

Fleas, Blood and Plague!

Viveka Vadyvaloo, Benjamin Burrows

Washington State University, Pullman, USA

95 -

Rickettsia-flea interactions associated with vector infection and transmission

Kevin Macaluso

Louisiana State University, Baton Rouge, USA

42 -

Vector Biology of Parasitic Lice

Lance Durden

Georgia Southern University, Statesboro, USA

157 -

Molecular adaptation of *Bartonella quintana* to its human and louse niches

Henriette Macmillan¹, David M. Dranow^{2,3}, Sally Lyons-Abbott^{2,4,5}, Gina M. Borgo¹, James W. Fairman^{2,3,6}, Stephanie J. Huezio¹, Donald D. Lorimer^{2,3}, Bart L. Staker^{2,4}, Robin Stacy^{2,4}, Stephanie Abromaitis^{1,7}, Michael J. Trnka⁸, Thomas E. Edwards^{2,3}, Peter J. Myler^{2,4,9}, Jane E. Koehler¹

¹Microbial Pathogenesis and Host Defense Program, and Division of Infectious Diseases, Department of Medicine, University of California, San Francisco, CA, USA. ²Seattle Structural Genomics Center for Infectious Disease, Seattle, WA, USA. ³Beryllium Discovery Corp, Bainbridge Island, WA, USA. ⁴Center for Infectious Disease Research, Seattle, WA, USA. ⁵Novo Nordisk Inc, Seattle, WA, USA. ⁶Roche Sequencing Solutions, Santa Clara, CA, USA. ⁷California Department of Public Health, Richmond, CA, USA. ⁸Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, USA. ⁹Departments of Global Health, and Biomedical Informatics & Medical Education, University of Washington, Seattle, WA, USA

Round Table Discussion

Lunch on your own

Time: 12:20 - 14:00

Date: 9th June 2019

Poster Session 1 Preview

Time: 12:20 - 14:00

Date: 9th June 2019

Location: Zia Ballroom

Session 3: Microbiome

Chair: Jeanne Salje & Irene Newton

Time: 14:00 - 15:45

Date: 9th June 2019

Location: Anzasazi Ballroom

158 -

Friends or foes? Investigating how ticks navigate diverse microbial communities

Seemay Chou

University of California, San Francisco, San Francisco, USA

152 -

Microbiome of *Leptotrombidium* Mite Vectors of Scrub Typhus

Loganathan Ponnusamy¹, Alexandra Wilcox², R. Michael Roe¹, Silas Davidson³, Anthony Schuster³, Allen Richards⁴, Steven Meshnick², Charles Apperson¹

¹North Carolina State University, Raleigh, USA. ²University of North Carolina, Chapel Hill, USA. ³Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ⁴Naval Medical Research Center, Silver Spring, USA

156 -

Reproductive Parasitism: The Pieces, Players and Shifting Paradigm

Joseph Gillespie¹, Timothy Driscoll², Victoria Verhoeve¹, Mark Guillotte¹, Kristin Rennoll-Bankert¹, M. Sayeedur Rahman¹, John Beckman³, Darren Hagen⁴, Christine Elisk⁵, Kevin Macaluso⁶, Abdu Azad¹

¹University of Maryland School of Medicine, Baltimore, USA. ²West Virginia University, Morgantown, USA. ³Auburn University, Auburn, USA. ⁴Oklahoma State University, Stillwater, USA. ⁵University of Missouri, Columbia, USA.

⁶Louisiana State University, Baton Rouge, USA

Round Table Discussion

Poster Session 1

Time: 15:45 - 17:30

Date: 9th June 2019

Location: Zia Ballroom

150 -

Mechanisms of pathogen entry into tick cells

Hanan Baggar¹, Jessie Ujcz², Debra Alperin¹, Susan Noh^{1,2}

¹Washington State University, Pullman, USA. ²USDA-ARS-Animal Disease Research Unit, Pullman, USA

148 -

Sequence of a novel *Anaplasma marginale* genome determined with next generation PacBio sequencing technology

Kyle Hoffman¹, Sammuell Shahzad¹, Michael Calcutt¹, Kelly Brayton², Mark Foecking¹, Roger Stich¹

¹University of Missouri, Columbia, USA. ²Washington State University, Pullman, USA

147 -

Immune intervention targeting a tick vector of *Anaplasma marginale*

Bill Stich¹, Kyle Hoffman¹, Sammuell Shahzad¹, Chelsea Zorn¹, Sara Scott¹, Sathaporn Jittapalapong², Gayle Johnson¹, Patrick Pithua³, Guoquan Zhang¹

¹University of Missouri, Columbia, USA. ²Kasetsart University, Bangkok, Thailand. ³Virginia Tech, Blacksburg, USA

145 -

Rickettsial organisms associated with ticks collected from Missouri elk

Sammuel Shahzad¹, Zhenyu Shen¹, Kelly Straka², Elizabeth Daugherty¹, Dana Thompson¹, Michael Zhang¹, Jeffery Mitchell¹, Shuping Zhang¹, Roger Stich¹

¹University of Missouri, Columbia, USA. ²Missouri Department of Conservation, Columbia, USA

144 -

Identification of distinct *Anaplasma marginale* genotype repertoires in different herds within the same beef cattle operation

Tippawan Anantatat, Brandt Skinner, Emily Reppert, Kathryn Reif

Kansas State University, Manhattan, USA

142 -

OPT4e: a tool for predicting T4SS effector proteins

Zhila Esna Ashari, Michael Dodd, Shira Broschat, Kelly Brayton

Washington State University, Pullman, USA

141 -

Identification of Type IV Secretion System Effectors of *Anaplasma phagocytophilum*

Deirdre Fahy¹, Curtis Nelson², Jason Park¹, Michael Dodd¹, Nicole Burkhardt², Lisa Price², Shira Broschat¹, Daniel Voth³, Jonathan Oliver², Ulrike Munderloh², Kelly Brayton¹

¹Washington State University, Pullman, USA. ²University of Minnesota, Minneapolis, USA. ³University of Arkansas for Medical Sciences, Little Rock, USA

139 -

***Galleria mellonella* infection reveals *Coxiella* effectors important for control of host tolerance**

Shawna Reed, Emerson Crabill, David Arteaga, Jorge Meneses, Craig Roy

Yale University School of Medicine, New Haven, USA

138 -

***Ehrlichia chaffeensis* transposon mutagenesis library**

Ying Wang¹, Andy Alhassan^{1,2}, Ulrike Munderloh³, Roman Ganta¹

¹Center of Excellence for Vector-Borne Diseases (CEVBD), Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA. ²Department of Pathobiology, School of Veterinary Medicine, St. George's University, West Indies, Grenada. ³Department of Entomology, University of Minnesota, St. Paul, MN, USA

136 -

Importance of host cytoskeleton protein vimentin to *Anaplasma phagocytophilum* intracellular development

Curtis Read, Chelsea Cockburn, Jason Carlyon

Virginia Commonwealth University, Richmond, USA

133 -

Preliminary Characterization of a *Bartonella quintana* Zinc Uptake Regulator

Callum Howett, Joanna MacKichan

Victoria University of Wellington, Wellington, New Zealand

132 -

The First *Ehrlichia ruminantium* Experimental Infection Study in North American Sheep

Arathy Nair¹, Huito Liu¹, Ying Wang¹, Giselle Cino², Jamie Henningson², Roman Ganta¹

¹Center of Excellence for Vector-Borne Diseases, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, USA. ²Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, USA

131 -

Identification of Mammalian Host Responses During *Rickettsia rickettsii* Infection

Jessica Towe¹, Victoria Verhoeve², Sean Riley³, Timothy Driscoll¹

¹West Virginia University, Morgantown, USA. ²University of Maryland School of Medicine, Baltimore, USA. ³Louisiana State University, Baton Rouge, USA

129 -

Identification of small novel immunoreactive *Ehrlichia* proteins that contain transmembrane domains and conformation-dependent antibody epitopes

Tian Luo, Jignesh Patel, Xiaofeng Zhang, Thangam-Sudha Velayutham, Jere McBride

University of Texas Medical Branch, Galveston, USA

128 -

Initial Development of Vaccine Candidates against Spotted Fever Rickettsioses

Ilirjana Hyseni¹, Sagar Gaikwad¹, Nicole Burkhardt², Ulrike Munderloh², Rong Fang¹

¹Department of Pathology, University of Texas Medical Branch, Galveston, USA. ²Department of Entomology, University of Minnesota, St. Paul, USA

127 -

Three's company: a pair of divergent *Wolbachia* spp. coinfecting the cat flea, *Ctenocephalides felis*

Joseph Gillespie¹, Victoria Verhoeve¹, Kevin Macaluso², Abdu Azad¹, John Beckmann³, Timothy Driscoll⁴

¹University of Maryland, Baltimore, USA. ²Louisiana State University, Baton Rouge, USA. ³Auburn University, Auburn, USA. ⁴West Virginia University, Morgantown, USA

125 -

The role of Annexin a2 in spotted fever group *Rickettsia* infection-associated microhemorrhage in the brain

Zhengchen Su, Qing Chang, Thomas Shelite, Yakun Liu, Jie Xiao, Aleksandra Drelich, Xi He, Soong Lynn, William Russell, Bin Gong

University of Texas Medical Branch , Galveston, USA

124 -

Cell-Specific Regulation of Autophagy by MYD88 during Fatal *Ehrlichia* Infection

Abdeljabar El Andaloussi¹, Muhamuda Kader², Mohammed Halloul¹, Nahed Ismail¹

¹University of Illinois at Chicago, Chicago, USA. ²University of Pittsburg, Pittsburg, USA

123 -

Role of lipid droplets in prostaglandin E2 production during *Coxiella burnetii* infection.

Morgan Harrison, Minal Mulye

Marian University College of Osteopathic Medicine, Indianapolis, USA

121 -

Type IV Secretion-dependent Inhibition of NF-κB Transcriptional Networks by *Coxiella burnetii*

Elizabeth Case, Erin van Schaik, Saugata Mahapatra, James Samuel

Texas A&M Health Science Center, Bryan, USA

120 -

Detection of zoonotic bacterial pathogens in various hosts in the Mnisi community, Mpumalanga, South Africa using a microbiome sequencing approach

Agatha O. Kolo¹, Nicola E. Collins¹, Kelly A. Brayton^{1,2}, Lucille H. Blumberg³, John A. Frean³, Jeanette M. Wentzel⁴, Cory A. Gall², Marinda C. Oosthuizen¹

¹Vectors and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa. ²Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, USA. ³National Institute for Communicable Diseases, Johannesburg, South Africa. ⁴Hans Hoheisen Wildlife Research Station, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

119 -

Humoral immunity mediated by human monoclonal antibodies, protects against *Ehrlichia chaffeensis* through novel intracellular mechanisms

Thangam Sudha Velayutham¹, Sandeep Kumar¹, Xiaofeng Zhang¹, David H. Walker¹, Gary Winslow², James E. Crowe³, Jere W. McBride¹

¹University of Texas Medical Branch, Galveston, USA. ²Upstate Medical University, Syracuse, USA. ³Vanderbilt University Medical Center , Nashville, USA

118 -

Macrophage heterogeneity correlates with the pathogenesis of ehrlichiosis.

Edson R.A. Oliveira¹, Mohamed Haloul^{1,2}, Muhamuda Kader³, Tyler Tominello³, Jakob Z. Wells³

¹University of Illinois at Chicago, Chicago, USA. ²Children's Cancer Hospital Egypt 57357, Cairo, Egypt. ³University of Pittsburgh, Pittsburgh, USA

117 -

Thailand-Myanmar mil-mil collaborative surveillance program for rickettsiosis

Jariyanart Gaywee¹, Han Tin Aung², Wuttikorn Rodkvamtook¹, Myo Thant², Kamonwan Siriwanthanakul¹, Kyaw Soe², Maneerat Somsri¹, Chanokaun Timhaiphon¹, Yutthapong Sudsawat¹, Kiatisak Somsri¹, Weera Boonsome¹, Min Kramyoo¹, Thitisak Niratsai³, Tin Maung Hlaing², Pramote Imwattana¹

¹Armed Forces Research Institute of Medical Science, Bangkok, Thailand. ²Defense Services Medical Research Centre, Nay Pyi Taw, Myanmar. ³Royal Thai Army Medical Department, Bangkok, Thailand

116 -

Disruption of ORP1L (Oxysterol Binding Protein 1 Long) in murine alveolar macrophages attenuates *Coxiella burnetii* intracellular growth

Baleigh Schuler, Stacey Gilk

Indiana University School of Medicine, Indianapolis, USA

114 -

***Orientia tsutsugamushi* modulates cellular levels of NF-κB p105**

Tanaporn Wangsanut, Haley Adcox, Sarika Gupta, Jason Carlyon

Virginia Commonwealth University, Richmond, USA

113 -

Identification of small RNAs expressed *in vitro* by *Bartonella bacilliformis* under a variety of conditions that simulate the human host and sand fly vector

Shaun Wachter¹, Rahul Raghavan², Michael Minnick¹

¹The University of Montana, Missoula, USA. ²Portland State University, Portland, USA

110 -

***Coxiella burnetii* infections in mice: Clinical and immunological responses to contemporary genotypes**

Rachael Priestley, Cody Smith, Halie Miller, Gilbert Kersh

Centers for Disease Control and Prevention, Atlanta, USA

109 -

Investigation of a Novel Uncharacterized Rickettsiales in *R. amblyommatis* isolate Ac37

Nicole Burkhardt, Roderick Felsheim, Lisa Price, Timothy Kurtti, Ulrike Munderloh

University of Minnesota, St. Paul, USA

108 -

***Anaplasma marginale* outer membrane protein vaccine candidates are conserved in North American and South African strains**

Paidashe Hove^{1,2}, Kelly Brayton^{3,1}, Junita Liebenberg⁴, Alri Pretorius⁴, Marinda Oosthuizen¹, Susan Noh⁵, Nicola Collins¹

¹Vectors and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa. ²Agricultural Research Council–Biotechnology Platform, Pretoria, South Africa.

³Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, USA. ⁴Agricultural Research Council–Onderstepoort Veterinary Research, Pretoria, South Africa. ⁵Animal Disease Research Unit, Agricultural Research Service, US Department of Agriculture, Pullman, USA

107 -

Development of Highly Sensitive and Specific Acute Diagnostic Tests for Tick-Borne Spotted Fever Rickettsioses

Rong Fang¹, Jeremy Bechelli¹, Claire Smalley¹, José A. Oteo², Yingxin Zhao^{3,4,5}, Allan R. Brasier^{3,4,5}, David H. Walker¹

¹Department of Pathology, University of Texas Medical Branch (UTMB) , Galveston, USA. ²Centre of Rickettsiosis and Arthropod-Borne Diseases, Hospital San Pedro-CIBIR, Logroño, La Rioja, Spain. ³Department of Internal Medicine, UTMB, Galveston, USA. ⁴Institute for Translational Sciences, UTMB, Galveston, USA. ⁵Sealy Center for Molecular Medicine, UTMB, Galveston, USA

106 -

Serological and clinical features of patients with PCR-proven infection by *Anaplasma phagocytophilum*

J. Stephen Dumler¹, Gary P. Wormser², Johan S. Bakken³

¹Uniformed Services University of the Health Sciences, Bethesda, USA. ²New York Medical College, Valhalla, USA.

³University of Minnesota, Duluth, Duluth, USA

104 -

The analysis of plasma metabolites in mice survived against the *Orientia tsutsugamushi* infection

Sangho Choi¹, Min-Gyu Yoo², Jae-yon Yu¹, Hye-Ja Lee², Seong Beom Cho³, Sung Soon Kim¹, Hyuk Chu¹

¹Division of Bacterial Disease Research, Center for Infectious Diseases Research, Korea National Institute of Health, Cheongju, Korea, Republic of. ²Division of Endocrine and Metabolic Disease, Center for Biomedical Sciences, Korea National Institute of Health, Cheongju, Korea, Republic of. ³Division of Bio-Medical Informatics, Center for Genome Science, Korea National Institute of Health, Cheongju, Korea, Republic of

103 -

Surveillance on Rickettsial Disease in the country of Georgia

Giorgi Chakhunashvili, Ekaterine Zhgenti, Roena Sukhiashvili, David Tsereteli, Paata Imnadze, Ekaterine Javidze
National Center for Disease Control and Public Health, Tbilisi, Georgia

93 -

Requests for rickettsial disease diagnostics over 6 years in Sri Lanka; room for improvement?

Ranjan Premaratna, Wijesinghe Bandara, Ravini Premaratna, Nilmini Chandrasena
Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

91 -

Evidence of exposure to spotted fever group rickettsioses (SFGR) and *Orientia tsutsugamushi* (OT) in Gampaha and Colombo districts in Western Sri Lanka

Ranjan Premaratna, Wijesinghe Bandara, Ravini Premaratna, Nilmini Chandrasena
Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

Dinner on your own

Time: 17:30 - 19:30

Date: 9th June 2019

Session 4: Mentoring Session

Time: 19:30 - 21:00

Date: 9th June 2019

Location: Anasazi Ballroom

MONDAY

Breakfast

Time: 7:00 - 8:00

Date: 10th June 2019

Location: Cava

Session 5: Ecology and Epidemiology

Chair: Kevin Macaluso & Maria Galletti

Time: 8:00 - 10:05

Date: 10th June 2019

Location: Anzasazi Ballroom

102 -

Flea-borne rickettsial disease outbreaks: what are the causative agents?

Allen Richards

Uniformed Services University of the Health Sciences, Bethesda, USA

88 -

Tick-borne encephalitis virus and *Rickettsia* spp. in ticks collected from birds in Hesse, Germany

Michael Wimbauer¹, Silke Wölfel², Michael Bröker³, Sabine Schaper⁴, Ramona Riess⁴, Gerhard Dobler⁴, Lidia Chitimia-Dobler⁴

¹Independent scientist, Bad Wildungen, Germany. ²amedes MVZ for Laboratory Medicine and Microbiology, Fürstentfeldbruck, Germany. ³Independent scientist, Marburg, Germany. ⁴Bundeswehr Institute of Microbiology, Munich, Germany

16 -

The coinfection with *Ehrlichia minasensis*, *Anaplasma marginale* and a new *Anaplasma* genotype is not associated with anemia in beef cattle in the Brazilian Pantanal, an endemic area for bovine trypanosomiasis in South America

Inalda Ramos¹, Ana Cláudia Calchi¹, Diego Zanatto¹, Victória de Mello¹, Bruna Horta¹, Júlia Tasso¹, Heitor Herrera², Rosângela Machado¹, Marcos Andre¹

¹Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (FCAV/UNESP), Jaboticabal, Brazil.

²Universidade Católica Dom Bosco (UCDB), Campo Grande, Brazil

64 -

Ehrlichiosis in Adair County, Missouri from 2014 to Present

Deborah Hudman

AT Still University, Kirksville, USA

76 -

Flea-borne *Rickettsia* in Pennsylvania

Brooke Coder¹, Marcie Lehman¹, Richard Stewart¹, Ju Jiang², Allen Richards², Christina Farris², Alison Fedrow^{1,2}

¹Shippensburg University, Shippensburg, USA. ²Naval Medical Research Center, Silver Spring, USA

137 -

A Preliminary Plan For Instituting A State-Wide Tick Management Program

Lauren Maestas

Delaware Fish & Wildlife, Newark, USA

43 -

The Case for Passive Surveillance: Tick Testing and Military Populations

Robyn Nadolny, Ellen Stromdahl

Army Public Health Center, Aberdeen Proving Ground, USA

146 -

Confirmation of *Anaplasma phagocytophilum* in South Africa by multilocus sequencing

Agatha Kolo¹, Nicola Collins¹, Mamohale Chaisi², Lucille Blumberg³, John Frean³, Marinda Oosthuizen¹, Kelly Brayton^{4,1}

¹University of Pretoria, Pretoria, South Africa. ²National Zoological Gardens of South Africa, Pretoria, South Africa.

³National Institute for Communicable Diseases, Johannesburg, South Africa. ⁴Washington State University, Pullman, USA

Break

Time: 10:05 - 10:45

Date: 10th June 2019

Location: Zia Ballroom

Session 6: Vaccines/Infection, Diagnosis & Treatment

Chair: Steve Dumler & Katelynn Doiron

Time: 10:45 - 11:45

Date: 10th June 2019

Location: Anzasazi Ballroom

47 -

***Neorickettsia finleia* sp. nov., a bacterium that causes Potomac horse fever**

Omid Teymounejad¹, Hannah Bekebrede¹, Luis Arroyo², John Baird², Yasuko Rikihisa¹

¹The Ohio State University, Columbus, USA. ²University of Guelph, Guelph, Canada

61 -

High-throughput screening of modulators of cellular calcium metabolism as potential drugs against rickettsia-induced microvascular dysfunction

Yuri Kim¹, Emily Clemens², Jinyang Wang¹, Dennis Grab², J. Stephen Dumler²

¹Henry M. Jackson Foundation for Advancement of Military Medicine, Bethesda, USA. ²Uniformed Services University of the Health Sciences, Bethesda, USA

134 -

***Anaplasma marginale* infection of *Dermacentor andersoni* through an *in vitro* tick feeding system**

Rubikah Vimonish, Wendell Johnson, Glen Scoles, Susan Noh, Massaro Ueti

USDA, Pullman, USA

143 -

Comparison of chlortetracycline and oxytetracycline treatment regimens to clear bovine anaplasmosis

Kathryn Reif, Tippawan Anantatat, Michael Kleinhenz, Emily Reppert, Johann Coetzee

Kansas State University, Manhattan, USA

80 -

Vaccine-mediated protection against pulmonary Q fever in three animal models.

Anthony Gregory¹, Erin van Schaik¹, Kasi Russell-Lodrigue², Alycia Fratzke¹, James Samuel¹

¹Texas A&M Health Science Center, Bryan, USA. ²Tulane National Primate Research Center, Covington, USA

Optional Taos Trip

Time: 12:00 - 17:00

Date: 10th June 2019

Dinner on your own

Time: 17:00 - 19:00

Date: 10th June 2019

Session 7: Host-Pathogen-Vector Interactions

Chair: Anders Omsland & Minal Mulye

Time: 19:30 - 21:30

Date: 10th June 2019

Location: Anzasazi Ballroom

35 -

CBU_1276 is Required for Intracellular Replication and Resistance to Oxidative Stress by *Coxiella burnetii*, the Causative Agent of Q fever

Mebratu A. Bitew¹, Janine Hofmann², David P. De Souza³, Nadeeka K. Wawegama², Hayley J. Newton⁴, Fiona M. Sansom²

¹Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, The University of Melbourne, Melbourne, Australia. ²Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, The University of Melbourne,, Melbourne, Australia. ³Metabolomics Australia, Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, , Melbourne, Australia. ⁴Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, , Melbourne, Australia

37 -

***Coxiella burnetii* Subversion of the Macrophage Unfolded Protein Response**

Katelynn Doiron, Marissa Fullerton, Daniel Voth

University of Arkansas for Medical Sciences, Little Rock, USA

79 -

Expanded specificity of an antibacterial tick effector Dae2 enables inhibition of host and environmental microbes.

Beth Hayes, Atanas Radkov, Seemay Chou

UCSF, San Francisco, USA

97 -

The relevance of *A. phagocytophilum* adhesin binding domains to *in vivo* infection

Waheeda Naimi, Jacob Gumpf, Ryan Green, Richard Marconi, Jason Carlyon
Virginia Commonwealth University, Richmond, USA

45 -

Mice lacking interferon signaling as robust models to investigate *Rickettsia parkeri* pathogenesis and host response

Thomas Burke, Patrik Engström, Matthew Welch
University of California, Berkeley, Berkeley, USA

100 -

Modifications of outer membrane protein B protect *Rickettsia parkeri* from ubiquitylation

Patrik Engstrom, Matthew Welch
University of California, Berkeley, Berkeley, USA

115 -

An *Orientia tsutsugamushi* effector modulates cellular levels of the MHC-I transactivator NLRC5

Haley Adcox, Kyle Rodino, Jason Carlyon
Virginia Commonwealth University, Richmond, USA

TUESDAY

Breakfast

Time: 7:00 - 8:00

Date: 11th June 2019

Location: Cava

Session 8: T4 Effectors

Chair: Dan Voth & Joseph Gillespie

Time: 8:00 - 9:50

Date: 11th June 2019

Location: Anzasazi Ballroom

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Identification and characterization of T4SS substrates in *Wolbachia pipientis*

Irene Newton, Kathy Sheehan, Danny Rice, MaryAnn Martin

Indiana University, Bloomington, USA

14 -

A conserved, potential nucleomodulin in *Rickettsia* species

Hema P. Narra, Sandhya R. Golla, Abha Sahni, Krishna M. Sepuru, Sanjeev K. Sahni

University of Texas Medical Branch, Galveston, USA

40 -

A *Rickettsia typhi* phosphatidylinositol 3-kinase, RT0135, is an *rvh* type IV secretion system effector that modulates plasma membrane phosphoinositide metabolism during invasion and subverts cellular autophagy to establish its intracellular niche

Oliver H. Voss¹, Joseph J. Gillespie¹, Stephanie S. Lehman², Sherri A. Rennoll¹, Magda Beier-Sexton¹, M. Sayeedur Rahman¹, Abdu F. Azad¹

¹Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, USA. ²Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, USA

52 -

Inhibition of *Ehrlichia chaffeensis* Infection by Cell-permeable Bicyclic Peptides that Bind Ehrlichial Type IV Secretion Effector Etf-1

Mingqun Lin, Amritendu Koley, Wenqing Zhang, Dehua Pei, Yasuko Rikihisa

The Ohio State University, Columbus, OH, USA

99 -

Modulation of NF- κ B signalling by a *Coxiella burnetii* eukaryotic-like effector protein

Melanie Burette, Julie Allombert, Ghizlane Maarifi, Sebastien Nisole, Karine Lambou, Matteo Bonazzi

CNRS, Montpellier, France

112 -

A small RNA that regulates pyrimidine and methionine metabolism and possibly type IV secretion system effector CvpD is necessary for establishing *Coxiella burnetii*'s intracellular niche during infection

Shaun Wachter¹, Matteo Bonazzi², Kyle Shifflett¹, Abraham Moses³, Rahul Raghavan³, Michael Minnick¹

¹The University of Montana, Missoula, USA. ²CNRS, Universite Montpellier, Montpellier, France. ³Portland State University, Portland, USA

89 -

Initial structural characterisation of the DNA binding domain of Anka from *Anaplasma phagocytophilum*

Ian Cadby¹, Andrew Lovering¹, Patrick Moynihan¹, J. Stephen Dumler²

¹University of Birmingham, Birmingham, United Kingdom. ²Uniformed Services University of the Health Sciences, Bethesda, USA

Break

Time: 9:50 - 10:30

Date: 11th June 2019

Location: Zia Ballroom

Session 9: Omics

Chair: Joao Pedra & Shaun Wachter

Time: 10:30 - 12:00

Date: 11th June 2019

Location: Anzasazi Ballroom

77 -

Dual RNA sequencing reveals insights into the biology of *Orientia tsutsugamushi*

Bozena Mika-Gospodorz¹, Suparat Giengkam², Alexander Westermann¹, Jantana Wongsantichon², Suthida Chuenklin², Piyanate Sunyakumthorn³, Radoslaw Sobota⁴, Jorg Vogel¹, Lars Barquist¹, Jeanne Salje⁵

¹Helmholtz Institute for RNA-based Infection Research, Wurzburg, Germany. ²Mahidol Oxford Research Unit, Bangkok, Thailand. ³Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ⁴A*Star, Singapore, Singapore. ⁵Public Health Research Institute, Rutgers University, Newark, USA

50 -

Transcriptomic Profiling of Pulmonary Gene and lncRNA Expression in a Murine Model of *Rickettsia conorii* Infection

Imran Chowdhury, Hema Narra, Abha Sahni, Kamil Khanipov, Yuriy Fofanov, Sanjeev Sahni

University of Texas Medical Branch, Galveston, TX, USA

3 -

Mechanisms of establishing infection in *Wolbachia*

Amelia Lindsey, Irene Newton

Indiana University, Bloomington, USA

60 -

Histone H3 deacetylation in ex vivo human neutrophils infected by *Anaplasma phagocytophilum*

Jianyang Wang¹, J. Stephen Dumler²

¹Henry M. Jackson Foundation, Bethesda, USA. ²Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, USA

130 -

Now This Party's Jumping! Sequence, assembly and annotation of the cat flea *Ctenocephalides felis* (Bouché) genome.

Timothy P. Driscoll¹, Victoria I. Verhoeve^{1,2}, Joseph J. Gillespie², Mark L. Guillotte², Kristen E. Rennoll-Bankert², M. Sayeedur Rahman², Darren Hagen³, Christine G. Elsik^{4,5,6}, Kevin R. Macaluso⁷, Abdu F. Azad²

¹Department of Biology, West Virginia University, Morgantown, West Virginia, USA. ²Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, USA. ³Department of Animal and Food Sciences, Oklahoma State University, Stillwater, Oklahoma, USA. ⁴Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA. ⁵Division of Plant Sciences, University of Missouri, Columbia, Missouri, USA. ⁶MU Informatics Institute, University of Missouri, Columbia, Missouri, USA. ⁷Vector-borne Disease Laboratories, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, USA

5 -

MALDI-TOF MS for rapid identification of arthropod vectors of rickettsial agents in Europe and Africa

Philippe Parola, Maureen Laroche

University Hospital Institute Méditerranée Infection, Marseille, France

Lunch

Time: 12:00 - 14:00

Date: 11th June 2019

Location: Cava

Poster Session 2

Time: 12:00 - 14:00

Date: 11th June 2019

Location: Zia Ballroom

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Molecular Detection of *Rickettsia* in American Dog Ticks Collected Along the Platte River in South Central Nebraska

Brandon Luedtke¹, Julie Shaffer¹, Estrella Monrroy¹, Corey Willicott¹, Travis Bourret²

¹University of Nebraska-Kearney, Kearney, USA. ²Creighton University School of Medicine, Omaha, USA

101 -

Dynamic Gene Expression of Cat Flea-Derived Salivary Gland-Secreted Factors during *Rickettsia felis* Infection

Monika Danchenko, Hanna Laukaitis, Kevin Macaluso

Vector-borne Disease Laboratories, Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, USA

98 -

Role of *Sca4* in the Dissemination and Transmission of *Rickettsia parkeri* in *Amblyomma maculatum*

Chanakan Suwanbongkot¹, Chanida Fongsaran¹, Ingeborg Langohr², Rebecca L. Lamason³, Kevin Macaluso¹

¹Vector-Borne Disease Laboratories, Department of Pathobiological Science, School of Veterinary Medicine, Louisiana State University, Baton Rouge, USA. ²Department of Pathobiological Science, School of Veterinary Medicine, Louisiana State University, Baton Rouge, USA. ³Department of Biology Massachusetts Institute of Technology, Massachusetts, USA

94 -

Comparative proteomics of different developmental stages of the Rickettsiales *Orientia tsutsugamushi*

Jantana Wongsantichon¹, Radoslaw Mikolaj Sobota², Jeanne Salje^{1,3,4}

¹Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

²Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore. ³Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. ⁴Public Health Research Institute, Rutgers Biomedical and Health Science, Newark, New Jersey, USA

92 -

***Anaplasma marginale* infection dynamics in an endemic region**

Roberta Koku^{1,2}, David Herndon³, Johannesty Avillan^{1,2}, James Futse^{4,1}, Susan M. Noh^{3,2,1}

¹Paul G. Allen School of Global Animal Health, Pullman, USA. ²College of Veterinary Microbiology and Pathology, Washington State University, Pullman, USA. ³United States Department of Agriculture, Pullman, USA. ⁴College of Basic and Applied Science, University of Ghana, Accra, Ghana

90 -

First detection and characterization of *Coxiella burnetii* in ixodid ticks from Somalia

Dimitrios Frangoulidis¹, Claudia Kahlhofer¹, Ahmed Shire Said², Yassir Adam Shuaib³, Lidia Chitimia-Dobler¹

¹Bundeswehr Institute of Microbiology, Munich, Germany. ²College of Veterinary Medicine, East Africa University, Bosaso, Somalia. ³College of Veterinary Medicine, Sudan University of Science and Technology, Hilat Kuku, Khartoum North, Sudan

86 -

Transcriptome Profiling of Inflammasome-Mediated Responses in Macrophages to *R. australis* Infection

Ilijana Hyseni¹, Steven Widen², David H. Walker¹, Rong Fang¹

¹Department of Pathology, University of Texas Medical Branch, Galveston, USA. ²Sealy Center for Molecular Medicine, Galveston, USA

84 -

Profile of Serum Exosomal MicroRNAs in Mice Infected with *Orientia tsutsugamushi*

Le Jiang¹, Belinskaya Tatyana¹, Zhiwen Zhang¹, Teik-Chye Chan¹, Wei-Mei Ching^{1,2}, Chien-Chung Chao^{1,2}

¹Naval Medical Research Center, Silver Spring, USA. ²Uniformed Services University of the Health Sciences, Bethesda, USA

82 -

Elimination of the *Coxiella burnetii* QpH1 Plasmid

Paul Beare¹, Heather Miller¹, Daniel Voth², Robert Heinzen¹

¹Rocky Mountain Labs, NIH, NIAID, Hamilton, USA. ²Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, USA

81 -

Identifying molecular determinants of *B. burgdorferi* transmission in ticks

Anne Sapiro¹, Beth Hayes¹, Amy Lyden², Emily Crawford², Seemay Chou^{1,2}

¹University of California, San Francisco, San Francisco, CA, USA. ²Chan Zuckerberg Biohub, San Francisco, CA, USA

78 -

Genetic characterization of a new strain of *Ehrlichia chaffeensis* from an *Amblyomma tenellum* tick from South Texas.

Esteban Arroyave^{1,2}, Bethany Quade³, Nicole L. Mendell¹, Lucas S Blanton³, Donald H. Bouyer¹

¹Department of Pathology, The University of Texas Medical Branch, Galveston, USA. ²Research Group on Veterinary Sciences "Centauro", Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellin, Colombia. ³Department of internal medicine, The University of Texas Medical Branch, Galveston, USA

75 -

The *Anaplasma phagocytophilum* Adhesin Asp14 Exploits Host Cell Surface Protein Disulfide Isomerase Activity to Promote Infection

Ryan Green¹, Waheeda Naimi¹, Lee Oliver Jr.¹, Nathaniel O'Bier¹, Rebecca Martin¹, Richard Marconi¹, Daniel Conrad¹, Jaehyung Cho², Jason Carlyon¹

¹Virginia Commonwealth University School of Medicine, Richmond, USA. ²University of Illinois College of Medicine, Chicago, USA

74 -

Clinical characterization of *Orientia tsutsugamushi* Boryong strain in rhesus macaques (*Macaca mulatta*)

Sujitra Tayamun¹, Manutsanun Sumonwiriya¹, Noppon Popruk¹, Rawiwan Im-erbsin¹, Kesara Chumpolkulwong¹, Matthew D. Wegner¹, Luis A. Lugo-Roman¹, Piyanate Sunyakumthorn¹, Nam-Hyuk Cho²

¹Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ²Seoul National University College of Medicine, Seoul, Korea, Republic of

73 -

***Rickettsia philipii* Infection in Mammalian Cells and Expression of Unique Genes Encoded by a Chromosome Insert**

Marina Eremeeva, Sunmisola Olade

Georgia Southern University, Statesboro, USA

71 -

Surveillance of Tick-borne Pathogens in Louisiana

Sabrina Valdes¹, Sean Simonson², Julius Tonzel², Christine Scott-Waldron², Gary Balsamo², Britton Grasperge¹, Kevin Macaluso¹

¹Vector-borne Disease Laboratories, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA. ²Louisiana Office of Public Health, Infectious Disease and Epidemiology Section, New Orleans, LA, USA

70 -

A genome-wide siRNA screen to study host factors involved in the early stages of infection by *Orientia tsutsugamushi*

Yanin Jaiyen¹, Porncheera Chusorn², Potjanee Kanjanapiboon³, Mathupanee Oonsivilai¹, Somponnat Sampattavanich², Jeanne Salje^{1,4,5}

¹Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand. ²SICORE for Systems Pharmacology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand. ³Department of Biomedical Engineering, Faculty of Engineering, Mahidol University, Bangkok, Thailand. ⁴Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. ⁵The Public Health Research Institute Center, New Jersey Medical School – Rutgers, The State University of New Jersey, Newark, USA

69 -

***Anaplasma phagocytophilum* Infection Involves Cholesterol-driven Association of NPC1 and Flotillin 2**

Weiyan Huang, Qingming Xiong, Mingqun Lin, Yasuko Rikihisa
The Ohio State University, Columbus, OH, USA

68 -

Role of microRNAs in apoptosis inhibition during *Coxiella burnetii* infection

Madhur Sachan, Rahul Raghavan
Portland State University, Portland, USA

67 -

The Effect of Extended Laboratory Propagation on The Genome and Virulence of *Orientia tsutsugamushi*

Suthida Chuenklin¹, Suwittra Chaemchuen¹, Jantana Wongsantichon¹, Elizabeth Batty^{1,2,3}, Piyanate Sunyakumthorn⁴, Jeanne Salje^{1,3,5}

¹Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand. ²Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom. ³Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. ⁴Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ⁵Public Health Research Institute, Rutgers Biomedical and Health Sciences, Newark, New Jersey, USA

63 -

Susceptibility of *Rickettsia rickettsii* to Tigecycline

Bethany Quade, Nicholas Wilson, David Walker, Lucas Blanton
University of Texas Medical Branch, Galveston, USA

59 -

Developing an ELISA as a diagnostic assay for the detection of *Rickettsia felis*

Hanna Laukaitis¹, Kelsey Legendre^{1,2}, Kevin R. Macaluso¹

¹Louisiana State University, Baton Rouge, USA. ²Auburn University, Auburn, USA

58 -

Inhibition of *Ehrlichia chaffeensis* Infection by Intracellular Nanobodies that Bind *Ehrlichia* Type IV Secretion Effector Etf-1

Wenging Zhang, Mingqun Lin, Libo Hou, Jeffery Lakritz, Yasuko Rikihisa
The Ohio State University, Columbus, OH, USA

55 -

New labelling approaches lead to new insights into the biology of Rickettsiales bacteria

Sharanjeet Atwal^{1,2,3}, Jeanne Salje^{1,2,3}

¹University of Oxford, Oxford, United Kingdom. ²Mahidol Oxford Tropical Research Unit, Bangkok, Thailand. ³Public Health Research Institute, Rutgers University, New Jersey, USA

54 -

Characterizing Early Stages of Human Alveolar Infection by the Q Fever Agent, *Coxiella burnetii*

Amanda Dragan¹, Richard Kurten², Dan Voth¹

¹University of Arkansas for Medical Sciences, Little Rock, USA. ²Arkansas Children's Hospital Research Institute, Little Rock, USA

53 -

Persistence of *Coxiella burnetii* following an abortion storm on a small Texas goat farm

Cody Smith, Rachael Priestley, Cara Cherry, Halie Miller, Gilbert Kersh
Centers for Disease Control and Prevention, Atlanta, USA

51 -

Searching Algorithm for Type IV Effector proteins (S4TE) 2.0: improved tools for type IV effectors prediction, analysis and comparison in proteobacteria

Christophe Noroy^{1,2,3}, Damien Meyer^{1,2}

¹CIRAD, UMR ASTRE, 97170 Petit-Bourg, Guadeloupe, France. ²ASTRE, Université de Montpellier, CIRAD, INRA, Montpellier, France. ³Université des Antilles, 97159 Pointe-à-Pitre, Guadeloupe, France

49 -

The metalloenzyme ENO1 is ubiquitinated by *Ehrlichia chaffeensis* TRP120

Bing Zhu¹, Clayton E. Kibler¹, Jennifer Y. Wang^{1,2}, Jere W. McBride^{1,3,2,4,5,6}

¹Departments of Pathology, University of Texas Medical Branch, Galveston, TX,, Galveston, USA. ²Neuroscience, Cell Biology & Anatomy, University of Texas Medical Branch, Galveston,TX,, Galveston, USA. ³Departments of Microbiology & Immunology, University of Texas Medical Branch, Galveston,TX,, Galveston, USA. ⁴Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston,TX,, Galveston, USA. ⁵Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston,TX,, Galveston, USA. ⁶Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston,TX,, Galveston, USA

48 -

Sequencing protocol modification and validation for increased efficiency of *Rickettsia* species determination in clinical specimens

Yan Zeng, Ida Chung, Arlyn Gleaton, Cecilia Kato

Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch, Atlanta, USA

44 -

Development and validation of real-time PCR assays to detect *Rickettsia typhi* in clinical specimens

Ida Chung, Yan Zeng, Arlyn Gleaton, Cecilia Kato

Centers for Disease Control and Prevention, Atlanta, USA

41 -

Does it work? Effects of permethrin-treated uniforms on tick submissions to a passive tick surveillance program

Robyn Nadolny, Cory Casal, Scott Haynes, Ellen Stromdahl

Army Public Health Center, Aberdeen Proving Ground, USA

39 -

The putative auto-transporter proteins ScaD and ScaE from *Orientia tsutsugamushi* are sufficient to mediate adherence to target mammalian cells

Daniel A Garza, Juan J Martinez

Vector-Borne Disease Laboratories, Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, USA

38 -

Identification pipeline of Anaplasmataceae type IV effectomes.

Stéphanie Silou^{1,2,3}, Damien F. Meyer^{1,2}

¹CIRAD, UMR ASTRE, 97170 Petit-Bourg, Guadeloupe, France. ²ASTRE, Univ Montpellier, CIRAD, INRA, Montpellier, France. ³Université des Antilles, 97159 Pointe-à-Pitre, Guadeloupe, France

36 -

Developing a recombinant *Coxiella* O-antigen

Sumita Roy, Alice Cross, Nicholas Harmer

Living System Institute, University of Exeter, EXETER, United Kingdom

34 -

Urine metabolite analysis of experimental animal with *Orientia tsutsugamushi* infection

Sangho Choi¹, Jae-yon Yu¹, Sung Soon Kim¹, Min-Gyu Yoo², Hye-Ja Lee², Seong Beom Cho³, Hyuk Chu¹

¹Division of Bacterial Disease Research, Center for Infectious Diseases Research, Korea National Institute of Health, Cheongju, Korea, Republic of. ²Division of Endocrine and Metabolic Disease, Center for Biomedical Sciences, Korea National Institute of Health, Cheongju, Korea, Republic of. ³Division of Bio-Medical Informatics, Center for Genome Science, Korea National Institute of Health, Cheongju, Korea, Republic of

33 -

Distribution of *Orientia tsutsugamushi* in *Leptotrombidium* mites, scrub typhus vector and reservoir

Piyanate Sunyakumthorn¹, Piyada Linsuwanon¹, Sirima Wongwairot¹, Manutsanun Sumonwiriya¹, Matthew Wegner¹, Luis Lugo-Roman¹, James Jones¹, Brett Swierczewski¹, Elizabeth Wanja¹, Silas Davidson¹, Nicholas Day^{2,3}, Carl Mason¹, Daniel Paris^{2,4,5}

¹Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ²Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand. ³Centre for Tropical Medicine and Global Health, Oxford, United Kingdom. ⁴Nuffield Department of Clinical Laboratory Sciences, Oxford, United Kingdom. ⁵Swiss Tropical and Public Health Institute, Basel, Switzerland

32 -

Dynamics of the cytokine storm in experimental scrub typhus non-human primate model (Rhesus macaques) intradermally inoculated with *O. tsutsugamushi* Karp and Gilliam strain

Manutsanun Sumonwiriya¹, Raviwan Im-erbsin¹, Sirima Wongwairot², Susanna J Dunachie^{2,3}, Matthew D Wegner¹, Luis A Lugo-Roman¹, James W Jones¹, Allen L Richards^{4,5}, Carl J Mason¹, Nicholas P.J Day^{2,3}, Daniel H Paris^{2,6,7}, Piyanate Sunyakumthorn¹

¹Department of Veterinary Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. ²Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand. ³Center for Tropical Medicine and Global Health, Oxford, United Kingdom. ⁴Department of Viral & Rickettsial Diseases, Naval Medical Research Center, Maryland, USA. ⁵Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Maryland, USA. ⁶Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland. ⁷Faculty of Medicine, University of Basel, Basel, Switzerland

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The occurrence of *Anaplasma phagocytophilum* in cattle after grazing in the Republic of Korea

Kyoung-Seong Choi, Ji-Hyoung Ryu

Kyungpook National University, Sangju, Korea, Republic of

30 -

Nitric Oxide Reduces *Rickettsia rickettsii* Viability

Liam Fitzsimmons, Tina Clark, Ted Hackstadt

National Institute of Allergy and Infectious Disease, Hamilton, USA

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Regulation of CX3CL1 expression in human microvascular endothelial cells during *Rickettsia rickettsii* infection by microRNA-424

Abha Sahni, Hema Narra, Sanjeev Sahni

University of Texas Medical Branch, Galveston, USA

27 -

Genetic Heterogeneity of the *scaA-scaF* Autotransporter Genes of *Orientia tsutsugamushi*

Gregory A Dasch¹, Munegowda C. Koralur², Arunachalam Ramaiah³

¹Centers for Disease Control and Prevention, Atlanta, GA, Georgia. ²Centers for Disease Control and Prevention, Atlanta, GA, USA. ³University of California, Irvine, Irvine, CA, USA

26 -

Characterization of an emergent large plaque variant of *Rickettsia rickettsii* Sheila Smith

Adam Nock, Tina Clark, George Glidden, Ted Hackstadt

NIAID, Hamilton, USA

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Degradation of Tumor Suppressor FBW7 by *Ehrlichia chaffeensis* TRP120-Mediated Ubiquitination Promotes Infection

Jennifer Y. Wang, Bing Zhu, Jere W. McBride

University of Texas Medical Branch, Galveston, USA

24 -

The identification of *Coxiella burnetii* virulence-associated genes in *Galleria mellonella*

Georgie Metters¹, Claudia Hemsley¹, Joann Prior², Isobel Norville², Richard Titball¹

¹University of Exeter, Exeter, United Kingdom. ²Defence Science and Technology Laboratory, Salsbury, United Kingdom

23 -

Elucidating and exploiting O-antigen biosynthesis for Q fever vaccine development

Alice Cross¹, Sumita Roy¹, Joann Prior^{1,2,3}, Nicholas Harmer¹

¹Living Systems Institute, College of Life & Environmental Sciences, University of Exeter, Exeter, United Kingdom.

²Defence Science and Technology Laboratory, Porton Down, Salisbury, United Kingdom. ³London School of Hygiene & Tropical Medicine, London, United Kingdom

21 -

Vaccination with recombinant Asp14 and OmpA of *Anaplasma phagocytophilum* in lambs gave serological responses, but ineffective protection against challenge.

Erik Georg Granquist¹, Sveinung Eskeland¹, Francy Liliana Crosby², Kari Lybeck³, Anthony F Barbet², Per-Eric Lindgren⁴, Stig Tollefsen³, Peter Wilhelmsson⁴, Shokouh Makvandi-Nejad³, Snorre Stuen⁵

¹Norwegian University of Life Sciences, Oslo, Norway. ²University of Florida, Gainesville, USA. ³Norwegian Veterinary Institute, Oslo, Norway. ⁴Linköping University, Linköping, Sweden. ⁵Norwegian University of Life Sciences, Sandnes, Norway

20 -

Molecular Detection of *Anaplasma* spp. in *Xenarthra* in Brazil

Ana Calchi¹, Mário Alves², Juliana Vultão³, Bruna de Oliveira², Camila Luba², Gabriel Massocato², Danilo de Souza², Arnaud Desbiez², Marta Teixeira³, Karin Werther⁴, Thiago da Silva⁴, Natalia Mendes⁴, Mariele de Santi⁴, Rosangela Machado⁴, Marcos Andre⁴

¹Faculdade de Ciências Agrárias e Veterinárias/Universidade Estadual Paulista, UNESP, Jaboticabal, Brazil. ²IPÊ – Instituto de Pesquisas Ecológicas - Projeto Bandeiras e Rodovias, Campo Grande, Brazil. ³Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil. ⁴Faculdade de Ciências Agrárias e Veterinárias/Universidade Estadual Paulista, Jaboticabal, Brazil

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Eukaryotic protein mimicry as an infection strategy for *Ehrlichia chaffeensis*

Madison Rogan, Caitland Byerly, Jere McBride

University of Texas Medical Branch, Galveston, USA

18 -

Development of *E. chaffeensis* TRP antigen detection assay for early diagnosis of human monocytotropic ehrlichiosis

Jignesh Patel¹, Xiaofeng Zhang¹, Jonathan Schmitz², Richard Willson³, David Walker¹, Jere W. McBride¹

¹University of Texas Medical Branch, Galveston, USA. ²Vanderbilt University, Nashville, USA. ³University of Houston, Houston, USA

17 -

Quantitative analysis of *Rickettsia rickettsii* in salivary glands of adult *Dermacentor variabilis* during the initial period of feeding

Michael L. Levin, Alyssa N. Snellgrove, Inna Krapivunaya, Shelby L. Ford

CDC, Atlanta, USA

15 -

Investigating the presence of *Rickettsia* spp. and *Yersinia pestis* in flea from the natural plague foci of Kazakhstan.

T. Yerubayev¹, T. Nurmakhanov¹, T. Meka-Mechenko¹, A. Abdirassilova¹, O. Yeskhojayev¹, A. Vilkova¹, D.

Ussenbekova¹, A. Richards², C. Farris², V. Motin³

¹M. Aikimbaev's Kazakh Scientific Center for Quarantine and Zoonotic Diseases (KSCQZD), Almaty, Kazakhstan.

²NMRC, Silver Spring, USA. ³UTMB, Galveston, USA

10 -

***Ehrlichia chaffeensis* activation of Notch signaling increases XIAP expression to inhibit intrinsic apoptosis**

LaNisha Patterson, Jennifer Wang, Jere McBride

The University of Texas Medical Branch, Galveston, USA

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Development of *Orientia tsutsugamushi* ScaA antigen-expressing recombinant non-replicative adenovirus virus vector for evaluation of protection against scrub typhus infection

Patricia Crocquet-Valdes, David Walker

University of Texas Medical Branch, Galveston, USA

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Distinct developmental stages during the intracellular life cycle of *Orientia tsutsugamushi*

Suparat Giengkam¹, Jantana Wongsantichon¹, Sharanjeet Atwal^{1,2}, Yanin Jaiyen¹, Graham Wright^{3,4}, Radoslaw M. Sobota^{4,5}, Jeanne Salje^{1,2,6}

¹Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand. ²Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, Oxford, United Kingdom. ³Skin Research Institute of Singapore, A*STAR, Singapore, Singapore. ⁴Science, Technology and Research (A*STAR), Singapore, Singapore.

⁵Institute of Molecular and Cell Biology, Functional Proteomics Laboratory, Agency for Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore. ⁶Public Health Research Institute, Rutgers the State University of New Jersey, Newark, USA

6 -

The importance of iron in *Coxiella burnetii* replication, viability and virulence

Savannah Sanchez, Anders Omsland

Washington State University, Pullman, USA

2 -

Characterization of a *Bartonella quintana* effector protein

Alvey Little, Joanna MacKichan

Victoria University of Wellington, Wellington, New Zealand

1 -

Systematic Review of Scrub Typhus Study Landscape: Protocol and Preliminary Literature Search Results

Kartika Saraswati^{1,2,3}, Brittany Maguire^{3,4}, Singh Sauman⁴, Nicholas Day^{2,3}, Philippe Guérin^{3,4}

¹Eijkman-Oxford Clinical Research Unit, Eijkman Institute for Molecular Biology, Jakarta, Indonesia. ²Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. ³Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

⁴Infectious Diseases Data Observatory (IDDO), Oxford, United Kingdom

Session 10: Pathogenesis I: *Coxiella*, *Orientia* and Ticks

Chair: Jason Carlyon & Mebratu Bitew

Time: 14:00 - 15:15

Date: 11th June 2019

Location: Anzasazi Ballroom

105 -

The *Coxiella burnetii* Type 4B Secretion System blocks host endosomal maturation

Dhritiman Samanta, Tatiana Clemente, Stacey Gilk

Indiana University School of Medicine, Indianapolis, USA

22 -

Hypoxia-induced citrate limitation results in *C. burnetii* containment in macrophages

Inaya Hayek¹, Fabian Fischer¹, Jan Schulze-Luehrmann¹, Katja Dettmer², Roland Lang¹, Peter J. Oefner², Stefan Wirtz³, Jonathan Jantsch⁴, Anja Lührmann¹

¹Mikrobiologisches Institut, Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Erlangen, Germany. ²Klinik für Innere Medizin I, Universitätsklinikum Regensburg, Regensburg, Germany. ³Medizinische Klinik 1, Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Erlangen, Germany. ⁴Institut für Klinische Mikrobiologie und Hygiene, Universitätsklinikum Regensburg, Regensburg, Germany

9 -

The *Coxiella burnetii* sterol modifying enzyme CBU1206 is critical for intracellular growth

Tatiana Mordente Clemente¹, Rochelle Ratnayake¹, Dhritiman Samanta¹, Paul Beare², Robert Heinzen², Stacey D. Gilk¹

¹Indiana University School of Medicine, Indianapolis, USA. ²Rocky Mountain Laboratories, Hamilton, USA

12 -

The function of ATGs in tick *Ixodes scapularis* autophagy in response to amino acid starvation

XINRU WANG, Timothy Kurti, Ulrike Munderloh

Department of Entomology, University of Minnesota, Saint Paul, USA

154 -

Biochemical Characterization of a Deubiquitinase Effector Protein from *Orientia tsutsugamushi*

Jason Berk¹, Haley Adcox², Christopher Lim¹, Yong Xiong¹, Jason Carlyon², Mark Hochstrasser¹

¹Yale University, New Haven, USA. ²Virginia Commonwealth University, Richmond, USA

Break

Time: 15:15 - 15:45

Date: 11th June 2019

Location: Zia Ballroom

Session 11: Pathogenesis II: *Ehrlichias* and *Rickettsias*

Chair: Stacey Gilk & Patrik Engstrom

Time: 15:45 - 17:00

Date: 11th June 2019

Location: Anzasazi Ballroom

11 -

***Ehrlichia chaffeensis* TRP120 Interacts with Notch Epidermal Growth Factor Domains to Activate Notch Signaling**

LaNisha Patterson, Jere McBride

The University of Texas Medical Branch, Galveston, USA

46 -

A biosafety level-2 dose-dependent lethal mouse model of spotted fever rickettsiosis

Andres F. Londono, Nicole L. Mendell, David H. Walker, Donald H. Bouyer

The University of Texas Medical Branch, Galveston, USA

13 -

Contribution of Host Lipid Metabolism to Pathogenicity of *Rickettsia* During Infection in Mammalian Cells

Paige Allen, Juan J. Martinez

Vector Borne Disease Laboratories, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, USA

87 -

Role of rickettsial O-antigen polysaccharide in parasitic lifecycle, pathogenesis, and protective immunity

Hwan Keun Kim¹, Ranjan Premaratna², Dominique Missiakas¹, Olaf Schneewind¹

¹University of Chicago, Chicago, USA. ²University of Kelaniya, Ragama, Sri Lanka

122 -

Understanding the molecular determinants of autoprocessing of APRc, the retropepsin-type protease from *Rickettsiae*

Pedro Curto^{1,2}, Marisa Lopes¹, Andreia Ferreira¹, Rui Cruz¹, Liliana Antunes¹, Isaura Simões^{1,2}

¹CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal. ²Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

Business Meeting

Time: 17:00 - 18:00

Date: 11th June 2019

Location: Anzasazi Ballroom

Gala Dinner

Time: 19:00 - 21:00

Date: 11th June 2019

Location: Cava

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Saturday, June 8, 2019
Abstracts

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Special Symposium

Chair: Janet Foley & Chris Paddock

62 -

Missing elements of natural history and ecology in Rickettsiology

Janet Foley

University of California, Davis, USA

Abstract

Some of the most elegant and foundational work in the ecology of infectious diseases was done during early investigation of Rocky Mountain spotted fever. Surveying biotic and abiotic contributors to pathogen persistence, infection, and disease are crucial early research steps, followed by increasingly rigorous documentation of pathogen diversity, host competence, and vectorial competence and capacity. The burgeoning field of disease ecology also considers theoretical aspects of disease and how they intersect traditional areas of interest in ecology including extinction, biological diversity, mathematical dynamics, and others. This presentation will offer a framework for synthesizing ecology and Rickettsiology, highlight where ecology and epidemiology intersect and diverge, and illustrate key needs to advance our understanding of the natural history and ecology of rickettsial diseases.

72 -

Rocky Mountain Spotted Fever and North Asian Tick Typhus: two diseases, the history, geography, diversity of the tick vectors, and common problems in the modern world.

Marina Ereemeeva

Georgia Southern University, Statesboro, USA

Abstract

Rocky Mountain Spotted Fever (RMSF) is the most prevalent tick-borne rickettsiosis in the New World; it is also the most malignant among known spotted fever rickettsioses. North Asian Tick Typhus (NATT) is a relatively mild illness which is broadly distributed throughout northern and central Asia, particularly Russia, China and Mongolia. The two diseases were among the earliest recognized tick-borne bacterial diseases, about thirty years apart first in Montana, USA and Far East of Russia, respectively. In both cases it took almost an additional 10 years to confirm the rickettsial etiology of these febrile illnesses and develop a formal description and recognition of their specific agents. Early recognition of the impact on human populations lead to both diseases becoming reportable in their respective countries. Over the years both diseases underwent several cycles of increasing or declining morbidity, most recently exhibiting continuously increasing trends similar to other tick-borne diseases. This presentation will compare novel and common ecological and epidemiological features defining the natural cycles of the two infections, RMSF due to *Rickettsia rickettsii* and NATT due to *R. sibirica*. This will include specific feature and gaps in information about the two etiological agents and their associations with various ticks and mammals. How this information can be applied toward evaluating and predicting the future environmental risks poses by these agents in humans and to better understand anthropogenic impacts on the dynamics of these diseases and their transmission will also be discussed.

65 -

What we know and what we don't know about the ecology of *Rhipicephalus sanguineus* transmitted rickettsias in the Mediterranean area

Philippe Parola

IHU Méditerranée Infection , Marseille, France

Abstract

The brown dog tick, *Rhipicephalus sanguineus* is known since the 1930s as the vector of *Rickettsia conorii conorii*, the agent of Mediterranean spotted fever, the most important and severe tick-borne disease occurring in Southern Europe and North Africa. Almost 100 years later, many basic questions regarding the relationships between the rickettsia and its tick vector are still unresolved. The life cycle of *R. conorii conorii* and the role of animal reservoirs is incompletely known. Another rickettsia, *R. massiliae* was first isolated in 1990 in *Rhipicephalus sanguineus* group ticks in southern France and described in 1993 as a new spotted fever group *Rickettsia*. It is a good example of a bacteria identified in ticks several years before its involvement in human diseases. To date, few human cases of *R. massiliae* infection have been reported. *R. massiliae* is a good example of a bacteria identified in ticks several years before its involvement in human diseases. However, as for *R. conorii conorii* many aspects of the ecology of this emerging agent are to be described. We discuss here several aspects that have been recently studied to better understand *Rh. sanguineus-rickettsia* relationships and ecology, as well as the remaining gaps.

155 -

The need for integrative approaches to deal with Rocky Mountain spotted fever in Sonora, Mexico

Gerardo Alvarez-Hernandez

University of Sonora, Hermosillo, Mexico

Abstract

Rocky Mountain spotted fever (RMSF) is among the most lethal of all infectious diseases in Mexico; the disease reemerged in the Northwest of the country during the early 2000's. Several factors are linked to its burden of morbidity and mortality, many of them triggered by socioeconomic lags and environmental determinants, interplaying in a complex causal chain difficult to address by traditional methodologies. Integrative interventions as proposed in the One Health approach should address the pivotal role of domesticated dogs and *Rhipicephalus sanguineus* sensu lato as drivers of epidemic levels of RMSF; additionally, socioecological models need to be implemented in similar regions as Mexico in order to build local capacity to deal with the multiple clinical, economic, public health, and social challenges related to the incidence of the disease. Moreover, ethical considerations and political willingness must be incorporated in the control and prevention of this life-threatening illness. Science has a fundamental role to alleviate the suffering caused by RMSF, by proposing and evaluating novel public health interventions, developing better diagnostic tools and therapeutic methods, and leading a shift in the public health paradigm of the disease. Describing some aspects of the RMSF epidemic in Sonora, I discuss the convenience of using interventions at multiple levels of organization to deal with a disease that, in Mexico, primarily affects socially vulnerable populations.

96 -

Deciphering the ecology of flea-borne spotted fever

Kevin Macaluso

Louisiana State University , Baton Rouge, USA

Abstract

Rickettsia felis was originally identified in the United States as a human pathogen in 1991 and is now associated with human infection in North and South America, Europe, Africa, Asia, and Oceania. Transmission of *R. felis* between cat fleas occurs by both horizontal and vertical routes; however, the roles of either transmission route in the spread of the agent are only beginning to be defined. Complicating the understanding of exposure risk is the presence of *Rickettsia typhi* and several *R. felis*-like organisms (RFLO's) with distinct genotypes that co-circulate with *R. felis* in flea populations. Furthermore, the apparent ability for *R. felis* to infect and be transmitted by other non-flea arthropods confounds the ecoepidemiology of *R. felis* rickettsiosis. Thus, our research objectives are to elucidate the biological and molecular mechanisms that are critical to rickettsial transmission by arthropods in order to better understand the epidemiology of flea-borne rickettsial diseases. We have determined that *R. felis* utilizes multiple mechanisms for rapid horizontal transmission between fleas and to other cofeeding arthropods, independent of a rickettsemic vertebrate host. Studies have also examined the potential for insect feces as an infectious source to vertebrate hosts. Combined, we have taken a multifaceted approach to decipher the vector and pathogen-associated factors essential to transmission and are assembling the components to identify the factors that contribute to *R. felis* infections.

151 -

The Ecology of Murine Typhus in the United States

Lucas Blanton

University of Texas Medical Branch, Galveston, USA

Abstract

Murine typhus is a flea-borne rickettsiosis caused by *Rickettsia typhi*. It is endemic to tropical and subtropical seaboard regions, where the primary reservoir (*Rattus* spp.) and their flea vectors (*Xenopsylla cheopis*) thrive. In the United States, the rat-flea cycle of transmission was all but eradicated with the strategic use of DDT on rat harborages. Despite the apparent eradication of murine typhus in most of the U.S. (5,401 cases in 1944 to fewer than 100 cases in 1956), the infection remained endemic to parts of southern California and South Texas. In these areas, an alternate reservoir-vector cycle, apparently involving opossums and *Ctenocephalides felis* fleas, appears to play a role in the transmission of *R. typhi* to humans. Indeed, in endemic areas, up to two thirds of opossums are seropositive for typhus group antibodies and up to 7% of their fleas are infected with *R. typhi*. Cases of murine typhus have been epidemiologically linked to cats, but the few *R. typhi*-infected fleas collected from these animals in the U.S. suggest that their role in the maintenance of *R. typhi* is limited. In the last decade there has been a resurgence of murine typhus in Texas with an apparent distribution that has marched into municipalities and counties north of its prior endemic range. Although the opossum-flea cycle of transmission is thought to play a role, the reason for the spillover into more northern areas is unknown. With the increasing incidence of murine typhus, understanding the changing ecology of flea-borne rickettsioses is necessary.

149 -

Ecology of *Amblyomma maculatum* and *Rickettsia parkeri*

Holly Gaff

Old Dominion University, Norfolk, USA

Abstract

Rickettsial tick-borne pathogens exhibit a wide range of prevalence rates in their tick vectors yet the drivers of this spatial and temporal heterogeneity are unknown. The recent range expansion of *Amblyomma maculatum* into the Mid-Atlantic area has led to population with much higher rates of *Rickettsia parkeri* than in the original range along the southern Gulf Coast. Through a multidisciplinary study, we are working to tease apart influences of environmental, physiological, and microbial factors on determination of these prevalence rates and thus human risk of tick-borne rickettsial infection. Our work thus far has found that *A. maculatum* is invading the Mid-Atlantic US using anthropogenically produced open, grassy habitat on the mainland as stepping-stones, and naturally occurring disturbed barrier island habitats as longer-term source populations from which ticks can disperse to ephemeral habitats on the mainland. The interplay of this invasion pattern and the resulting *R. parkeri* prevalence rates will be discussed.

56 -

Changing patterns and perceptions of tick-borne rickettsioses in western North America

Christopher Paddock

Centers for Disease Control and Prevention, Atlanta, USA

Abstract

During the 21st century, reported cases of tick-borne rickettsioses in the United States have followed a consistent upward trend, representing the steepest rise to the highest rates ever recorded. Since 2003, epidemic levels of Rocky Mountain spotted fever (RMSF) have emerged in the southwestern United States and in northern Mexico, where case fatality rates approach 30%-40%. The epidemiology and ecology of these outbreaks are distinct from classical RMSF and associated with large populations of free-roaming dogs that perpetuate enormous numbers of *Rhipicephalus sanguineus* sensu lato ticks in peridomestic settings. During the last 15 years, other tick-borne rickettsial pathogens have been identified in western North America, including *Rickettsia parkeri* and *Rickettsia 364D*. Cases of *R. parkeri* rickettsiosis, restricted previously to the southeastern and mid-Atlantic states and linked closely to the distribution of Gulf Coast ticks (*Amblyomma maculatum*), are now identified in southern Arizona and linked to previously unrecognized populations of ticks of the *A. maculatum* group in this region. A strain of *R. parkeri*, transmitted by *Dermacentor parumapertus* ticks and nearly identical to a pathogenic rickettsia identified recently in South America, has been recognized in Texas. *Rickettsia parkeri*-infected specimens of *D. parumapertus* and *A. maculatum* group ticks have also been found recently in northern Mexico. *Rickettsia 364D*, a species related closely to *Rickettsia rickettsii*, causes sporadic cases of disease in California, but is also likely to exist in *Dermacentor occidentalis* ticks in Mexico. Collectively, these observations reflect how the patterns and perceptions of historically recognized tick-borne rickettsioses evolve alongside the discovery of newly characterized pathogens.

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Session 1: Ticks

Chair: Ulrike Munderloh & Susan Noh

153 -

Tick Biology

Lorenza Beati

Georgia Southern University, Statesboro, USA

Abstract

In the last decade, many aspects of our knowledge of the biology of ticks have been revised and completed by new information. At the U.S. National Tick Collection, the focus of our research is taxonomy and population genetics. Therefore, the first part of this talk will deal with systematic questions that have proven to have an impact on our understanding of the eco-epidemiology of pathogen transmission. In particular, I will deal with the very different issues introduced by the study of the *Amblyomma cajennense* and by the *Amblyomma maculatum* group of species. These issues have revealed some incongruencies between molecular and morphological taxonomic approaches for species delimitations, that need to be addressed. In addition, I will present the result of the analysis of microsatellite markers of *Ixodes scapularis*, biparental nuclear markers that are offering a slightly different perspective on the population structure of this tick. During the second part of this talk, I will go through recent literature and focus on some of the topics that have raised my interest and have somewhat modified our perception of some aspects of tick biology.

85 -

Under Pressure: Stress Response at the Pathogen-Vector Interface

Kristin Rosche, Lindsay Sidak-Loftis, Shannon Allen, Brittany O'Keeffe, Dana Shaw

Veterinary Microbiology and Pathology, Washington State University, Pullman, USA

Abstract

Ixodes scapularis ticks can transmit at least seven pathogens relevant to human health. Why *I. scapularis* ticks are permissive to such a wide range of pathogens is not known. The arthropod immune system influences competence for pathogen colonization and transmission, but the molecular details of tick immunity remain vague. The noncanonical Immune Deficiency (IMD) pathway in ticks limits colonization of *Anaplasma phagocytophilum*, however, the molecular and cellular events preceding pathway activation are currently unknown. An attractive candidate for an upstream activation mechanism is the unfolded protein response (UPR), a highly conserved cellular reaction to endoplasmic reticulum (ER) stress. ER stress can be triggered by pathogens and *Anaplasma spp.* have been observed to closely associate with the ER in tick cells during intracellular infection. Furthermore, the mammalian analogue to the IMD pathway, the Tumor Necrosis Factor Receptor (TNFR) network, crosstalks with the UPR and propagates innate immune responses. These observations led us to investigate whether the UPR influences pathogen colonization in *I. scapularis* ticks. 14 UPR genes were found to be differentially induced in *A. phagocytophilum*-infected ticks. Transcriptionally knocking down or pharmacologically inhibiting the IRE1 α and ATF6 pathways, but not PERK, lead to increased *A. phagocytophilum* colonization both in *I. scapularis* cell lines and *in vivo* acquisition experiments. Taken together, these findings implicate a role for the UPR in vector competence of ticks.

126 -

Tick Immunity: Understanding the *Rickettsia parkeri* infection in *Amblyomma maculatum* tick interactions through innate immunity and redox signaling pathways.

Faizan Tahir

University of Southern Mississippi, Hattiesburg, USA

Abstract

Selenoproteins, incorporated from dietary selenium, plays an important role in immunity and inflammation responses due to its vital role in regulating reactive oxygen species and redox status in almost all tick tissues. Due to its importance, previous studies have been done to show that Selenophosphate synthetase 2 (SPS2), a homologue of selenophosphate synthetase (SelD) identified in mammals, is essential for selenoprotein biosynthesis. In addition to that, Relish, a homologue of nuclear factor-kappa B (NF- κ B), in the immune deficiency signaling pathway, regulates the expression of Microplusin, an antimicrobial peptide (AMP). In this study, we hypothesize that silencing of SPS2 and Relish will cause an increase in *Rickettsia parkeri* levels in infected *Amblyomma maculatum* ticks. To define the functional role of SPS2 and Relish in hematophagy and pathobiome colonization, an RNAi approach was utilized to deplete target genes expression in pathogen infected ticks. The transcriptional expression of target genes was confirmed in the knockdown tissues of both SPS2 and Relish. A significant decrease in replete weight, and a marked increase in distress in the host provided evidence for the critical role of target genes during feeding of knocked down ticks. A qPCR and 16s rRNA diversity assays showed that the gene-silenced ticks had significant increase in *R. parkeri* load than the control, proving that SPS2 and Relish play a role in the maintenance of tick pathobiome. Interplay between redox signaling and innate immunity pathways will be discussed in the context of tick-pathogen interactions.

135 -

Wiring tick signaling circuitry by a rickettsial pathogen

Girish Neelakanta

Center for Molecular Medicine, Department of Biological Sciences, Old Dominion University, Norfolk, USA

Abstract

The rickettsial pathogen *Anaplasma phagocytophilum* is the cause for the disease human anaplasmosis. This bacterium is transmitted to humans by the bite of infected black-legged ticks *Ixodes scapularis*. Upon transmission to humans, *A. phagocytophilum* primarily infects and survives in neutrophils. Being a vector-borne human pathogen, *A. phagocytophilum* has developed myriad number of strategies to survive both in neutrophils and ticks. The efforts from my laboratory are focused in the use of both ticks and tick cells to understand dynamics of *A. phagocytophilum*-tick interactions. Studies from my laboratory have provided evidence that *A. phagocytophilum* modulates arthropod organic anion transporting polypeptides (OATPs) and tryptophan pathway for its survival in ticks. By RNA interference and functional analysis, this study provides new evidences on the roles of several molecules in the tryptophan pathway critical for *A. phagocytophilum* survival in ticks. Transcription factor AP-1 was noted to be important in the regulation of kynurenine aminotransferase (KAT), a gene involved in the production of tryptophan metabolite xanthurenic acid (XA). Exogenous addition of XA induces OATP expression and *A. phagocytophilum* burden in both tick salivary glands and tick cells. New findings suggests that *A. phagocytophilum* modulate tick OATP-KAT signaling to fight against reactive oxygen species. In summary, this study not only provides detailed molecular evidences on the wiring of tick cell signaling by a rickettsial pathogen for its survival in the vector host but also lead for the development of better strategies to block transmission of this and perhaps other rickettsial species of medical importance.

66 -

Different features of *Amblyomma sculptum* and *Amblyomma aureolatum* midgut delineate their distinct susceptibility to infection with *Rickettsia rickettsii*

Nicolas Schröder¹, Flávia Ferreira², Larissa Martins², Maria Galletti^{2,3}, Daniel Pavanelo², José Ribeiro⁴, Marisa Farber⁵, Marcelo Labruna², Sirlei Daffre², Andrea Fogaça²

¹Institute of Biomedical Sciences/University of São Paulo, São Paulo, Brazil. ²University of São Paulo, São Paulo, Brazil. ³Centers for Disease Control and Prevention, Atlanta, USA. ⁴National Institute of Allergy and Infectious Diseases, Bethesda, USA. ⁵National Agricultural Technology Institute, Buenos Aires, Argentina

Abstract

Although *Amblyomma sculptum* and *Amblyomma aureolatum* are important vectors of *Rickettsia rickettsii* and belong to the same genus, *A. sculptum* is less susceptible to infection than *A. aureolatum*. Intriguingly, if the midgut (MG) of one specimen is infected, so are its salivary glands, pointing out the tick MG as an important barrier to infection. The transcriptome of the MG of *A. sculptum* and *A. aureolatum* showed that the majority of the coding sequences modulated by infection with *R. rickettsii*, including the immune factor gamma interferon inducible lysosomal thiol reductase (GILT), are upregulated in *A. sculptum* and downregulated in *A. aureolatum*. Specific dsRNAs for either GILT or green fluorescent protein (control) were injected to the hemocoel of adult *A. sculptum* ticks, which were then fed on infected rabbits. Only ticks injected with dsGILT acquired *R. rickettsii*, revealing this gene as an important factor to control infection. Tick microbiota is also known to exert an effect on establishment of pathogen infection. Therefore, the total number of bacteria within the tick MG was determined by qPCR using primers for the V2 variable region of the bacterial 16S rRNA. Data showed that the MG of noninfected *A. aureolatum* harbors around four orders of magnitude more bacteria than *A. sculptum*. In addition, while the bacterial load is significantly reduced in *R. rickettsii*-infected *A. aureolatum*, infection seems to exert no effect on the bacterial load of *A. sculptum*. The identification of the components of tick microbiota is underway.

Supported by: FAPESP, CNPq and CAPES.

Session 2: Fleas and Lice

Chair: Alison Fedrow & Mike Minnick

140 -

Fleas, Blood and Plague!

Viveka Vadyvaloo, Benjamin Burrows

Washington State University, Pullman, USA

Abstract

Bloodsucking fleas transmit several significant human pathogens including, *Yersinia pestis*, *Bartonella* spp and *Rickettsia* spp. In the case of *Y. pestis* that causes bubonic plague >280 flea species known to parasitize various rodent hosts have been implicated as vectors of this disease. Transmission of *Y. pestis* from fleas occurs predominantly by regurgitation of the bacteria from the flea foregut proventriculus, an organ central to the mechanism of transmission. The valve-like structure of the proventriculus and its rows of inward facing spines, are integral to its function to pump blood into the midgut, and macerate blood cells, during peristaltic digestion. Shortly after acquisition of the infected blood meal, *Y. pestis* autoaggregates within an exogenous reddish-brown matrix casing to form a biofilm characterized by a self-produced sticky extracellular polysaccharide matrix (EPS). The adherent biofilm fortifies attachment of *Y. pestis* to the foregut rendering it blocked and facilitating a pressurized reflux of the next fresh blood meal along with dislodged bacteria back into the bite-site. However, albeit consistently observed, a lesser defined process is the formation of the exogenous reddish-brown matrix. Here we demonstrate that this exogenous matrix forms and is primarily associated with the proventriculus by 24h post-infection during blood digestion. The exogenous matrix is specific to infection with *Y. pestis*, and not *Escherichia coli*, or following uninfected blood meal intake. In addition encasement of *Y. pestis* within the exogenous matrix is not biofilm-dependent, and correlates with protection from the early flea immune response to infection.

95 -

***Rickettsia*-flea interactions associated with vector infection and transmission**

Kevin Macaluso

Louisiana State University, Baton Rouge, USA

Abstract

Insect-borne bacterial pathogens are unique in their route of transmission from the vector to vertebrate hosts. Transmission can occur either through infectious saliva during arthropod feeding or by contact with infectious arthropod feces, or by both routes. Among the rickettsial pathogens, *Rickettsia felis* is an emerging agent associated with transmission to vertebrate hosts by cat fleas. Ongoing studies have identified multiple routes of horizontal transmission of *R. felis* and sequence analyses for multiple strains of *R. felis* have identified variations in plasmid quantity and content. Comparative analysis of vector infection and transmission for distinct rickettsial strains has failed to identify determinants for infection. However, analysis of *R. felis* transcription in distinct transmission states and sites have identified a common vector-specific adaptation, yet the role of these factors in transmission have yet to be defined. Consistent with other *Rickettsia*/vector relationships, rickettsial infection induces a transcriptional response in the flea and the strain-dependent induction and specific flea reaction is being characterized. Together, we seek to delineate the vector and pathogen-associated factors essential to transmission and to provide a platform to examine other flea-borne bacterial pathogens.

42 -

Vector Biology of Parasitic Lice

Lance Durden

Georgia Southern University, Statesboro, USA

Abstract

Worldwide, more than 5,000 species of parasitic lice have been described, including almost 600 species of obligately hematophagous sucking lice (Anoplura), all of which are ectoparasites of placental mammals. The entire life cycle (egg, 3 nymphal instars, adult male or female) of parasitic lice is spent on a homeothermic host. Because of their feeding behavior, sucking lice are more important vectors of pathogens and parasites than are chewing lice. Some sucking lice are apparently enzootic vectors of pathogens, including rickettsiae, between wild mammals, especially rodents. Humans can be parasitized by 3 kinds of sucking lice: head, body, and crab lice. Historically, the body louse has been implicated as the principal vector of the causative agents of epidemic typhus, trench fever, and louse-borne relapsing fever. More recently, it has also been implicated as a possible major vector of the causative agent of plague during historical outbreaks, especially the second pandemic. However, recent detection of some louse-borne pathogens in head lice has challenged the tenet that only body lice are vectors of human pathogens. Head and body lice are morphologically very similar but differ in their biology. Whether head and body lice represent different species, subspecies, ecotypes, or something else, continues to be debated. Analysis of several mitochondrial genes appears to separate head lice into 3 clades (A, B and C) and body lice into just one clade (A).

Molecular adaptation of *Bartonella quintana* to its human and louse niches

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Abstract

The general stress response (GSR) is a widely conserved response utilized by bacteria to survive under extreme environmental conditions. *Bartonella quintana* (BQ), occupies two distinct niches: the bloodstream of the human host (37°C; severely restricted hemin levels) or the gut of the human body louse vector (28°C; toxic hemin levels). We previously identified an extracytoplasmic function (ECF) sigma (s) factor, RpoE, which is involved in the GSR of BQ. When ingested by the body louse vector during a blood meal from the human host, BQ uniquely activates the GSR in response to the decreased temperature (28°C), and a BQ sensor histidine kinase (BQ-SHK) phosphorylates an anti-anti-s factor, PhyR. Phosphorylated PhyR then competes for, and removes, the anti-s factor NepR bound to the RpoE s factor, thus releasing RpoE. The RpoE ECF then binds RNA polymerase core enzyme, enabling transcription of the regulon necessary for survival under body louse conditions. To identify the structural basis of BQ GSR regulation in the body louse, we solved the crystal structures of the RpoE-NepR complex, the NepR-PhyR complex, and unbound, unphosphorylated PhyR. These crystal structures revealed a dramatic conformational change in PhyR after phosphorylation. The BQ-SHK null mutant was unable to activate the GSR in response to the 28°C stress signal of the body louse. Our data show that RpoE, BQ-SHK, and the conformational change of PhyR after phosphorylation all have a critical role in the adaptive response of BQ to low temperature stress in the body louse arthropod vector.

Session 3: Microbiome

Chair: Jeanne Salje & Irene Newton

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Friends or foes? Investigating how ticks navigate diverse microbial communities

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Abstract

Hard ticks serve as vectors for numerous human pathogens. Ticks also have the capacity to host a diverse range of non-pathogenic microbes, which could influence pathogen colonization through several mechanisms. We investigated whether the tick-borne Lyme disease pathogen *Borrelia burgdorferi* directly engages in interbacterial competition with bacteria in the midgut of wild, unfed *Ixodes scapularis* ticks using a combination of controlling sequencing methods and confocal microscopy. We found that the majority of examined ticks harbor limited internal microbial communities that are dominated by endosymbionts. Infectious challenges against *I. scapularis* ticks with *B. burgdorferi* or other tick-associated bacterial species showed that microbes that do not typically or stably colonize wild ticks are also significantly more toxic to their tick hosts. Furthermore, these guest microbes induce tick immune response signatures that are unique from *B. burgdorferi*, suggestive of a more antagonistic relationship. Interestingly, our study of an antimicrobial cell wall-degrading tick toxin known as Dae2 revealed higher levels of this enzyme in tick saliva compared to the tick gut, with biochemical specificity for common host skin-associated bacteria. We are currently testing the model that Dae2 acts primarily as a “gatekeeper” effector in saliva by killing bacteria that may enter the tick gut during feeding. Together, these results lead us to hypothesize that ticks have evolved important mechanisms to limit the number of microbes that can stably invade their own interior, creating a niche that *B. burgdorferi* and other tick-borne human pathogens can thrive in.

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Microbiome of *Leptotrombidium* Mite Vectors of Scrub Typhus

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Abstract

Scrub typhus is a severe mite-borne infection caused by *Orientia tsutsugamushi* (*Ot*), an obligate intracellular bacterium closely related to the genus *Rickettsia*. The disease occurs in Korea, Japan, throughout southern Asia, the Asian-Pacific region, and northern Australia where an estimated one billion people are at risk and approximately one million cases of scrub typhus are reported annually. The composition of bacteria populations in larvae, deutonymphs and adult males and females from laboratory colonies of *Leptotrombidium imphalum* that were infected as well as uninfected with *Ot* were investigated by high-throughput sequencing of the bacterial 16S rRNA gene. Remarkably, the bacterial microbiomes of infected adult females were dominated by sequences of *Ot* and an unidentified species of Amoebophilaceae, which together comprised 98.2% of bacterial sequences. The nearly full length of a cloned 16S rRNA gene sequence from infected female mites had 89 to 92% nucleotide identity with the Amoebophilaceae family, indicating that it is likely to be a species of a novel genus. α -diversity analyses revealed significant differences in species diversity between infected and uninfected adult mites. Beta diversity analyses also revealed that most of the variation in bacterial diversity across the samples could be attributed to infection with *Ot* and the Amoebophilaceae bacterium. These findings provide the basis for further studies to determine the influence of the novel Amoebophilaceae species on the bacterial microbiome and on vector susceptibility to and transovarial transmission of *Ot*.

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Reproductive Parasitism: The Pieces, Players and Shifting Paradigm

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Abstract

The ability of microbial parasites to drive their maternal inheritance in hosts by altering sexual reproduction (hereafter reproductive parasitism, RP) includes such processes as male-killing (MK), feminization, cytoplasmic incompatibility (CI), and parthenogenesis induction. Previous studies on certain fly species have elucidated factors underpinning MK (*Spiroplasma poulsonii* Spaid toxin) and CI (*Wolbachia* CinA/B and CidA/B toxin-antidote (TA) operons encoded within WO prophage). Via robust phylogenomics analysis of Spaid, CI-inducing TA modules and other putative RP-inducing proteins, we discerned that the intracellular mobilome (e.g., plasmids, phage and transposons) disseminates an intriguing assortment of RP-inducing genes across diverse intracellular bacteria. We proposed two hypotheses regarding the evolution of RP. First, we suggested *Wolbachia* nuclease (CinB) and deubiquitinase (CidB) toxins are derived from chimeric (CndB) toxins of ancestral *Rickettsia* TA operons, which inserted into WO prophage. Second, we posited that some arthropods have captured RP-inducing genes and incorporated them into their reproductive biology and/or immune arsenal against RP. Our sequencing and assembly of the cat flea genome unexpectedly supports these hypotheses. Firstly, we discovered a novel *Wolbachia* parasite, wCfeJ (close to Supergroups C and F), with a degraded WO prophage carrying a CinA/B module derived from a CndA/B operon. Secondly, the largest flea genome scaffold contains two loci (*cinA* and *cidA*) encoding antitoxin-like proteins most similar to CinA and CidA of *Wolbachia* Supergroups A and B. These findings broaden our understanding of RP origins, place RP-inducing TA operons within a host-parasite arms race, and inform efforts for utilizing reproductive parasites to control vector-borne pathogens.

Poster Session 1

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Mechanisms of pathogen entry into tick cells

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Abstract

Intracellular, tick borne bacterial pathogens cause diseases in humans and animals. Tools to prevent these diseases are limited in part due to our lack of understanding of the tick-pathogen interface. Within the tick, these pathogens must first enter the tick midgut cells while avoiding digestion. Digestion of the blood meal within the tick occurs intracellularly, rather than in the midgut lumen. Consequently, the midgut epithelial cells are designed for uptake of large volumes by a combination of processes, including non-receptor (macropinocytosis) and receptor-mediated endocytosis using clathrin and caveolin. How the pathogen leverages these processes to invade the midgut cells is unknown. While, references indicate intracellular, tick-borne pathogens enter the midgut via receptor-mediated endocytosis, there is no data supporting this claim. In this study we aim to determine the route by which *Francisella novicida* enters the tick midgut. *F. novicida*, used as a surrogate pathogen, completes replication and transmission within the tick similar to *A. marginale* and *A. phagocytophilum*. We determined there is an approximately 20% reduction in *F. novicida* entry in *Dermacentor andersoni* tick cells treated with monodansylcadaverine (MDC) or methyl- β -cyclodextrin (M β CD), indicating that host entry occurs via both clathrin-mediated and caveolin-mediated endocytosis. Additionally using EIPA, which inhibits macropinocytosis, there is a small, though not statistically significant reduction in bacterial uptake, suggesting a possible additional, though minor route of pathogen entry. The specificity of these inhibitors is not absolute, thus these findings will be confirmed using additional pathway inhibitors and primary tick midgut cultures.

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Sequence of a novel *Anaplasma marginale* genome determined with next generation PacBio sequencing technology

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Abstract

Tick transmission of infection is a complex interaction among the vector, vertebrate host and pathogen, resulting from extensive co-evolution among the organisms involved. Although it has been long understood that ticks transmit *A. marginale* and other rickettsial pathogens to livestock, anaplasma adaptations for transmission between mammalian hosts and tick vectors are not understood. One approach to this question is to compare genomes of phenotypically distinct strains to identify nucleotide sequences associated with tick transmissibility. Several *A. marginale* genomes have been sequenced to date, including the Florida (FL) which is reportedly non-transmissible by *Dermacentor* spp. ticks. The objectives of this study were to sequence, characterize and identify unique elements found in the Illinois (IL) strain genome, which is another non-tick-transmissible strain that is phenotypically distinct from the FL strain. The intact IL genome was purified from host blood that had been stored at -80 °C. Sequence analysis of an aaap-derived amplicon confirmed the identity of the infection as IL strain. The genomic DNA was sent to National Center for Genomic Resources (Santa Fe, NM) for next generation PacBio sequencing. The sequence data was used to assemble a preliminary IL genome, which was compared to published *A. marginale* genomes. Insertions of stop codons or frame shifts were confirmed with PCR and Sanger sequencing. Bioinformatic annotation was performed with Prokka, and IL genome annotation was manually compared to published *A. marginale* strain genomes. Further work will be described to identify sequences found only in IL or those shared amongst IL & FL.

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Immune intervention targeting a tick vector of *Anaplasma marginale*

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Abstract

The goal of anti-tick vaccine development is to develop sustainable interventions to decrease the incidence of tick-borne disease. Paradoxically, direct interference with the tick-pathogen interface is frequently overlooked as the *primary* criterion when screening for protective tick antigens. The central hypothesis of this project is that immunization with different tick tissue extracts will have different effects on the tick-borne pathogen, *Anaplasma marginale*, in *Dermacentor andersoni* ticks, as measured by acquisition, maintenance or transmission of the infection. Work to date includes i) measurement of performance parameters of ticks fed on calves immunized with salivary gland or midgut homogenates, to confirm feasibility of the *D. andersoni*-bovine host model, ii) comparative two-dimensional Western analysis of midgut-immune and salivary gland-immune bovine sera, iii) evaluation of different tick tissue homogenate treatments for immunization against *D. andersoni* and iv) adaptation of this host immunization approach to the tick-transmission model system of *A. marginale*. Results of the latter two studies are preliminary, with confirmatory trials pending. However, different effects were observed on *D. andersoni* feeding and fecundity after immunization with midgut or salivary gland homogenates. As expected, Western blot analysis confirmed that immunization with different homogenates induced antibodies that were cross-reactive as well as antibodies that were uniquely reactive with molecules associated with tick salivary glands or midgut. Experiments are underway to test the effects of immunization with these different midgut or salivary gland preparations on tick acquisition, maintenance and transmission of *A. marginale*.

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Rickettsial organisms associated with ticks collected from Missouri elk

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Abstract

The objective of this project was to identify ticks collected from 26 Missouri elk, from Fall 2015 through Spring 2016, and to screen these ticks for Anaplasmataceae, Rickettsiaceae and *Borrelia burgdorferi*. Dichotomous keys were used to identify adult stages of *Amblyomma americanum*, *Ixodes scapularis* and *Dermacentor albipictus*, and molecular tools are being used to speciate nymphal stages. A synthetic gene block, with previously validated primers for bacterial pathogens, was inserted into a plasmid and used as a positive control. Three different PCR assays were optimized and used to screen individual adult ticks and nymphs pooled according to collection date and host. Both strands of amplicons of expected size were sequenced, and then aligned with published sequences. Ten tick samples tested PCR-positive for tick-borne Anaplasmataceae, including three *Ixodes scapularis* which were positive for *Anaplasma phagocytophilum*. Fifty-six tick samples tested PCR-positive for Rickettsiaceae, including *Rickettsia amblyommatis* and *Rickettsia parkeri*; notably, several of these samples produced multiple amplicons, suggesting the possibility of co-infection with multiple Rickettsiaceae. Although three *I. scapularis* samples initially appeared to be PCR-positive for *Borrelia burgdorferi*, preliminary sequence analysis indicates that the expected *ospa* target sequence was not amplified. Amplicon sequence analysis is underway to identify posited rickettsial co-infections and to speciate nymphs. These results will aid in development of hypotheses with respect to the role of elk in the natural history of ticks and associated pathogens in Missouri.

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Identification of distinct *Anaplasma marginale* genotype repertoires in different herds within the same beef cattle operation

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Abstract

Anaplasma marginale, an obligate intracellular tick-borne rickettsial pathogen, is the causative agent of bovine anaplasmosis. In cattle, *A. marginale* infects red blood cells which can lead to severe anemia and, in some cases, death. Bovine anaplasmosis is conservatively estimated to cost the U.S. cattle industry at least \$300 million per year. The objective of this study was to evaluate the anaplasmosis infection prevalence and *A. marginale* genotype diversity in a privately-owned cattle operation in southeast Kansas with a history of anaplasmosis-related cattle deaths. This beef cattle, cow-calf operation maintains a combination of home-raised and purchased cattle in multiple herds. Blood samples were opportunistically collected from animals during routine pregnancy screening and *A. marginale* infection status and serological status was determined for individual animals using PCR and cELISA, respectively. From each herd, *A. marginale* genotype diversity was examined by cloning and sequencing the tandem repeat region of the Msp1a gene. Overall, the *A. marginale* infection prevalence in this cow-calf operation was 24%; however, home-raised animals maintained in separate herds from purchased cattle had a lower infection prevalence (16%) compared to purchased animals (44%). Over 50 *A. marginale* genotypes were identified with a greater diversity of genotypes detected in the purchased cattle herd compared to the home-raised herds. Incidental transmission of *A. marginale* between cattle herds and cattle operations is of significant concern among cattle producers; however, the maintenance of distinct *A. marginale* genotypes among multiple cattle herds within the same cow-calf operation indicates a low risk for incidental transmission among herds.

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OPT4e: a tool for predicting T4SS effector proteins

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Abstract

The Type IV Secretion System (T4SS) is utilized by pathogens to secrete effector proteins into host cells to manipulate host cell functions. Characterization of effectors is critical for understanding pathogenesis. Prediction of effector proteins is difficult due to a lack of conservation in effector repertoires and/or sequence between species. We have been working to develop a robust prediction tool. We started by performing a systematic study of published effectors and documenting all protein features that are associated with a protein being a T4 effector. Altogether, 1027 features were assembled and analyzed statistically to determine which correlated most with being a T4 effector. A reduced list of 370 features was further analyzed to determine how they performed to accurately predict effectors. To do this, one must have validated effectors, so this study was performed with *Legionella pneumophila*, and when used for de novo prediction, was able to predict >93% of the known T4 effectors. This strategy has been developed into a downloadable tool called Optimal-features Predictor for T4SS Effector proteins (OPT4e). The challenge now is to determine whether OPT4e will accurately predict T4 effectors for rickettsial agents. Applying OPT4e to *A. phagocytophilum* we predict 46-48 candidate effector proteins, depending on strain, and 26 effectors from *A. marginale*; predicting 90% of known effectors for these agents. Translocation of putative effectors is being tested using a heterologous reporter assay (see companion poster). Confirmed effectors can be easily incorporated into OPT4e, allowing for trainability and improved performance.

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Identification of Type IV Secretion System Effectors of *Anaplasma phagocytophilum*

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Abstract

Anaplasma phagocytophilum (Ap) is a zoonotic tick-transmitted obligate intracellular pathogen with a type IV secretion system (T4SS) capable of injecting bacterial effector proteins into the host cell. Since only a few Ap effectors have been identified to date our goal was to extend the repertoire. We have previously shown that *Anaplasma marginale* effectors can be translocated in a T4 dependent manner using a heterologous *Legionella pneumophila* reporter system. Ap Effectors were predicted based protein features including hydrophathy, homology to known effectors in other bacteria, and the presence of eukaryotic domains. Candidate genes were cloned into a Gateway® modified CyaA fusion vector. Following transformation of *Legionella* and infection of a macrophage cell line, translocated fusion proteins will result in cAMP production that can be assayed by ELISA yielding higher levels than non-translocated controls. Screening of ~30 candidate proteins has detected some proteins that appear to be translocated in a T4 dependent manner. Functional characterization, including subcellular localization, protein interactions, and mutant analysis will elucidate the role of these and other effectors in Ap growth and pathogenesis.

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***Galleria mellonella* infection reveals *Coxiella* effectors important for control of host tolerance**

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Abstract

Galleria mellonella larvae are widely used as a model for bacterial and fungal pathogenesis. To explore the role of *Coxiella burnetii* effector proteins in a multicellular host, we infected larvae with each of 68 isogenic Nine Mile Phase II transposon-disrupted strains and monitored survival for 10 days. As expected, *coxI*Δ, *cig57* and *cig2* disruption led to reduced virulence in the wax moth larvae. Eight different mutants were hypovirulent and three were hypervirulent when larval survival was compared to wild-type *Coxiella* NM II. Four of the seven hypovirulent strains, including *cig2::tn*, were also at a disadvantage in a competition assay with the wild-type strain. Replication was similar among all strains in whole larvae and HeLa cells, indicating that host death phenotypes resulted from differences in host tolerance or from failure of the bacteria to undergo multiple rounds of host cell infection. Two of the hypervirulent strains increased the IMD-dependent antimicrobial peptide response in *Drosophila* S2 cells. One predicted effector, YebC, appeared to be important for production of infectious progeny *in vitro* from HeLa cells. In addition, the *yebC::tn* strain expressing bacterial luciferase was less viable than wild-type during *in vivo* imaging of infected *Galleria* larvae. Thus, the larval infection model has revealed *Coxiella* effector genes that play an important role in animal virulence and in modulating conserved aspects of the host innate immune system. Lastly, exploratory experiments indicated that *Rickettsia parkeri* did not replicate or cause consistent death in *Galleria*.

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***Ehrlichia chaffeensis* transposon mutagenesis library**

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Abstract

Ehrlichia chaffeensis, a tick transmitted obligate intracellular rickettsial, is the agent of human monocytic ehrlichiosis and also causes canine ehrlichiosis. It is relatively challenging to perform genetic manipulations to identify genes contributing to pathogenesis. In recent studies, we described substantial advances in establishing targeted mutagenesis methods. The current study is focused on developing and characterizing a transposon mutagenesis library. Himar1 transposon-based random mutagenesis is reported by us earlier in creating insertion mutations in *E. chaffeensis* and by others in related pathogens. In particular, we previously reported mutations in 9 locations of *E. chaffeensis* genome and demonstrated their validity in defining the genes critical for the pathogen's persistent growth *in vivo* in reservoir and incidental hosts. We now generated a transposon mutagenesis library, which included 58 additional mutants. The mutations included 31 insertions within gene coding regions and 27 within the intergenic spacer sequences. RT-PCR analysis revealed transcriptional inactivation spanning the insertion sites of all 31 gene-coding regions. Several gene disruption mutations included in genes likely important for the bacterial pathogenesis. They included genes coding for two p28 outer membrane proteins (p28-Omp 1V and p28-1), 120 kDa immunodominant surface protein, queuine tRNA ribosyltransferase, efflux RND transporter permease subunit, adenosylmethionine 8-amino-7-oxononanoate transaminase, tRNA-methylthiotransferase MiaB, D-alanyl-D-alanine carboxypeptidase, dethiobiotin synthetase and several hypothetical proteins. Experiments are now under way in defining the impact of the insertion mutations for *E. chaffeensis* *in vivo* growth in tick and vertebrate hosts. These studies will likely provide insights about the functions of several uncharacterized genes of *E. chaffeensis*.

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Importance of host cytoskeleton protein vimentin to *Anaplasma phagocytophilum* intracellular development

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Abstract

Anaplasma phagocytophilum, the agent of human granulocytic anaplasmosis, is an obligate intracellular vacuole-adapted bacterium. One of the earliest infection events that occurs post-bacterial entry into tissue culture cells is irreversible wrapping of the *A. phagocytophilum*-occupied vacuole (ApV) by vimentin intermediate filaments. Pharmacologic blocking of vimentin polymerization pronouncedly reduces the *A. phagocytophilum* load. Whether ApV-vimentin interactions occur in the bacterium's primary mammalian host cell – the neutrophil – is unclear. Moreover, details outlining vimentin's relevance to *A. phagocytophilum* pathobiology are lacking. In this study, using immunofluorescence microscopy we confirmed that vimentin is recruited to the ApV in human neutrophils. As determined using qPCR and western blot analysis, the *A. phagocytophilum* load is significantly reduced during the first 24 h of infection in host cells in which vimentin levels have been knocked down using small interfering RNA. ApV expansion is impaired throughout the course of infection in vimentin knock-down cells. Yet, in vimentin knock-down cells *A. phagocytophilum* still expresses APH_0032 and APH_1235, which are an ApV membrane-localized effector and a bacterial outer membrane protein, respectively, that are only expressed during completion of the intracellular infection cycle. These data suggest that vimentin is important for optimal ApV expansion and bacterial replication, but is not absolutely required for progression of the *A. phagocytophilum* infection cycle.

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Preliminary Characterization of a *Bartonella quintana* Zinc Uptake Regulator

Callum Howett, Joanna MacKichan

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Abstract

Zinc is an essential trace element, required for the function of many proteins. Most living organisms, including bacterial pathogens, require zinc and have mechanisms to acquire it under limiting conditions. The mammalian host has evolved mechanisms to withhold trace metals, to slow or inhibit the growth of pathogens, which have evolved counter-mechanisms to acquire scarce nutrients, such as zinc. The louse-borne bacterial pathogen, *Bartonella quintana*, survives and persists for long periods of time, even months or years, in the bloodstream of the human host, despite of the relative absence of free zinc. The goal of our project is to understand how *B. quintana* senses and responds to low-zinc conditions. We identified a homologue of a Zinc Uptake Regulator in the *B. quintana* genome (Bq-Zur), based on the sequences of Zur homologues identified in other alpha-proteobacteria. We investigated the role of Bq-Zur through targeted mutagenesis and heterologous expression of Bq-Zur in *E. coli*. Interactions between purified Bq-Zur protein and promoter sequences were investigated by electrophoretic mobility shift assay, and a putative Zur-binding DNA motif was identified. These studies set the stage for understanding how *B. quintana* evades killing and clearance by host mechanisms, such as the neutrophil protein calprotectin, that function through zinc sequestration

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The First *Ehrlichia ruminantium* Experimental Infection Study in North American Sheep

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Abstract

Heartwater is a ruminant tick-borne disease caused by *Ehrlichia ruminantium*. It can cause up to 90% mortality rates in non-endemic regions, such as the US mainland. This Sub-Saharan African pathogen and one of its tick vectors, *Amblyomma variegatum*, are established in parts of the Caribbean. The presence of Heartwater in the Caribbean, coupled with availability of susceptible ticks on the US mainland, continue to threaten the US livestock industry. The USDA listed Heartwater under Tire 2 diseases of High Consequence and High Risk Foreign Animal Diseases and Pests. There were no studies documenting *E. ruminantium* infections in US ruminants. With a recent regulatory change, we could perform the first infection study in sheep. Six crossbred sheep were infected intravenously with blood stabilates representing six different pathogen strains; Gardel, Crystal springs, Nigeria, Malelane, Pokuase, and Highway. The sheep were monitored for 28 days for clinical signs, bacteremia, and for the antibody response. All animals exhibited lethargy and developed varying degrees of respiratory signs, while testing negative for the pathogen, but developed antibody responses. On necropsy, gross lesions were observed in lungs and trachea, which included variable degrees of pneumonia and edema. Hydropericarditis with moderate amounts of fluid was observed for Nigeria and Pokuase strains. Mild-to-moderate lymphoplasmacytic tracheitis, pneumonia, edema of aorta and myocardium and meningitis were also noted on histopathology in all animals. This is the first *E. ruminantium* infection study conducted in the USA and additional experiments to further define the disease pathogenesis are underway.

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Identification of Mammalian Host Responses During *Rickettsia rickettsii* Infection

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Abstract

An understanding of the interactions between intracellular pathogens and host cells is necessary in identifying molecular mechanisms that allow the pathogen to survive and cause disease in the host. During *Rickettsia rickettsii* infection, *R. rickettsii* adheres to the host cell using surface proteins, including rOmpB, and induces their engulfment. They quickly escape the phagosome and reproduce in the cytosol of the host cell before polymerizing the host actin and spreading to a new cell. Though various proteins involved in host cell invasion have been identified, these interactions and signaling pathways are still not fully understood. To explore this further, we have developed a mouse model to analyze the *in vivo* population dynamics of *Rickettsia rickettsii* and the host response during infection. In this study, we use RNA-seq to examine host and pathogen transcriptomes during infection. We further use pool-SEQ to compare population structures of *R. rickettsii* between *in vivo* and *in vitro* environments. We will leverage these results to identify host pathways targeted by *R. rickettsii* in different host cell types. From this understanding, we aim to provide further insight into the host responses involved during infection, and the impact these responses have on *R. rickettsii* success within host cells.

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Identification of small novel immunoreactive *Ehrlichia* proteins that contain transmembrane domains and conformation-dependent antibody epitopes

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Abstract

The known major immunoreactive proteins of *Ehrlichia chaffeensis* consist of ~10 protein orthologs with linear epitopes that include the well-characterized tandem repeat proteins (TRPs). However, this small group of defined immunoreactive proteins identified by Western immunoblotting likely represents an incomplete immunome of these pathogens. In this study, we combined innovative and high-throughput approaches including bioinformatic analysis to predict antigenicity, *in vitro* transcription and translation to express proteins in native conformation, and ELISA to identify a group of novel *E. chaffeensis* immunoreactive proteins with unknown function. The entire *E. chaffeensis* proteome (n=1156) was analyzed by the predictor of protein antigenicity, ANTIGENpro, which identified 250 proteins with a high antigenicity score (≥ 0.695). From hypothetical proteins (n=93) present in this highly antigenic group, we identified 5 proteins consistently and strongly immunoreactive with a panel of HME patient sera, including two at a level comparable to well-defined major immunoreactive TRPs. The majority (3/5) of these new immunoreactive proteins were small (< 22 kDa) and contained predicted transmembrane domains. Notably, the immunoreactivity of these proteins was predominately conformation-dependent as denaturation significantly affected antibody recognition. This study describes innovative approaches to identify immunoreactive proteins that expand the current number of known proteins in the *Ehrlichia* immunome, provides insight into the potential importance of small proteins and conformational dependent epitopes in immunity to *Ehrlichia*, and reveals new human and canine ehrlichiosis immunodiagnostic antigen prospects.

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Initial Development of Vaccine Candidates against Spotted Fever Rickettsioses

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Abstract

Rickettsiae are Gram-negative, vector-borne, obligately intracellular bacteria that potentially cause life-threatening infectious diseases in humans worldwide. Currently, no FDA-approved vaccine is available. In the present study, we aim to develop vaccine candidates against spotted fever rickettsioses. The growth and immunogenicity of a *R. parkeri* mutant, which contains the Himar1 transposase gene and a transposon segment comprising genes encoding mCherry co-expressed with the *aad* gene, is under evaluation as a vaccine candidate. *R. parkeri* mutant grew continuously while wild type *R. parkeri* were completely undetectable by quantitative real-time PCR in spectinomycin-treated Vero cells. *R. parkeri* mutant showed potent antigenicity as strongly as wild type by indirect immunofluorescence assay utilizing rabbit polyclonal antibodies directed against *R. massiliae*, a cross-reactive member of the spotted fever group rickettsiae. In addition, we have predicted the candidate epitopes for both B and T cells by multiple bioinformatics prediction methods based on the existing genome sequences of spotted fever group rickettsiae. The immunogenicity and safety of recombinant *R. parkeri* expressing the critical predicted epitopes will be determined. This proof-of-concept study will provide insight into developing a vaccine candidate for not only spotted fever rickettsioses but also multiple other tick-borne human pathogens.

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Three's company: a pair of divergent *Wolbachia* spp. coinfecting the cat flea, *Ctenocephalides felis*

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Abstract

Wolbachia (*Alphaproteobacteria*: Rickettsiaceae) parasites and symbionts are widely distributed intracellular bacteria that infect many arthropod species. They are maternally inherited and associated with a variety of host effects, both deleterious (reproductive manipulation) and beneficial (nutrient-provisioning). We have assembled complete genomes for two divergent *Wolbachia* spp. coexisting within the cat flea, *Ctenocephalides felis*, a medically important vector of human infectious disease agents, including *Rickettsia typhi* and *R. felis*. The first species (*wCfeT*) is basal to most described *Wolbachia* supergroups; it has a slightly enlarged genome (1.5 Mb), a largely-intact WO prophage, and a conserved biotin synthesis operon common to a diverse array of intracellular bacteria. *wCfeT* does not appear to be required for general *C. felis* fitness, since it is not present in other flea colonies; however, its significance and ecological distribution remain unknown. The second species (*wCfeJ*) is phylogenetically related to *Wolbachia* primary endosymbionts of filarial nematodes (supergroup C); it is characterized by a toxin-antitoxin gene pair (*cinAB*) associated with cytoplasmic incompatibility in a variety of arthropod endoparasites. rDNA (16S) sequence analysis indicates that both species have been associated with this *C. felis* colony since at least 2007, and we have further identified *wCfeT* in existing 16S-based surveys of natural flea populations. We hypothesize that *wCfeJ* is able to drive itself into *C. felis* populations using its toxin-antitoxin cassette, while *wCfeT* persists as a nutrient-providing endosymbiont. The effect of *wCfeT* and *wCfeJ* on the ability of *C. felis* to transmit *R. typhi* and *R. felis* is being investigated.

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The role of Annexin a2 in spotted fever group *Rickettsia* infection-associated microhemorrhage in the brain

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Abstract

Spotted fever group (SFG) rickettsioses are tick-borne diseases that occur worldwide and are caused by obligately intracellular bacteria of the genus *Rickettsia* (*R.*). The impact of SFG rickettsioses is high, due to the cost of treatment and the severe morbidity and mortality in case of misdiagnosis or inappropriate. *Rickettsia* targets host endothelial cells, weakens the endothelial barrier, and causes microvascular hyperpermeability and potentially fatal cerebral and pulmonary edema. In severe cases, perivascular hemorrhage may occur, especially in the hosts with poor health condition. However, the underlying mechanism remains largely unknown. We recently reported that endothelial Annexin a2 is a critical surface receptor for SFG rickettsiae to establish bacterial adhesion to vascular endothelial cells. Surprisingly, we found that Annexin a2 knockout (KO) mice consistently displayed brain microhemorrhage in response to *R. australis* infection compared to wild-type (WT) mice, indicating the essential role of Annexin a2 in protecting against rickettsial infection-associated central nervous system hemorrhage. We hypothesize that Annexin a2 plays a significant role in maintaining blood brain barrier function during rickettsia infection. To decipher the underlying mechanisms, brain tissues from infected and non-infected mice (KO and WT) were extracted and subjected to mass spectrometry. Preliminary analysis of proteomic data revealed a list of differentially expressed proteins. Functional enrichment analysis via DAVID database predicted a variety of significantly altered functional clusters that are associated with blood-brain barrier. Important functional clusters include cell-cell adherens junction and tight junction, vesicle-mediated transport, actin associated proteins, mitochondria, and calcium signaling pathway.

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Cell-Specific Regulation of Autophagy by MYD88 during Fatal *Ehrlichia* Infection

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Abstract

Autophagy is an important cell bioprocess responsible for eliminating and recycling non functional or degradative components or intracellular pathogens. MyD88 signaling is known to play a key role in regulation of several innate host responses including autophagy during infection with intracellular pathogens. Here, we investigated the contribution of MyD88 signaling to autophagy process during infection with virulent *Ehrlichia* in two target cells, macrophages and hepatocytes (HC) using MyD88^{-/-} mice. We isolated primary bone marrow derived macrophages (BMM) and primary HC from wild type (WT) and MyD88^{-/-} mice and examined autophagy flux following infection with highly virulent *Ixodes Ovatius Ehrlichia* (IOE). IOE-infected MyD88^{-/-} BMM and HC exhibited higher autophagy induction and flux compared to infected WT BMM. Increased autophagy in MyD88^{-/-} BMM and HC promoted bacterial replication compared to WT controls, which is consistent with prior studies showing that *Ehrlichia* hijack autophagy to obtain cell nutrients for their survival and replication. While autophagy induction inhibited inflammasome activation in MyD88^{-/-} macrophages, we did not detect significant inflammasome activation in either WT or MyD88^{-/-} HC. Mechanistically, MyD88-mediated inhibition of autophagy in BMM was due to mTORC1 activation. In contrast, MyD88-mediated inhibition of autophagy in HC was mTORC1-independent. Together, these data suggest cell-specific regulation of autophagy by MyD88 signaling.

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Role of lipid droplets in prostaglandin E2 production during *Coxiella burnetii* infection.

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Abstract

Coxiella burnetii, an obligate intracellular bacterium, causes potentially fatal endocarditis several years after initial infection suggesting the bacterium's ability to persist long-term in the host. Our overall goal is to determine the mechanisms *Coxiella* employs for its long-term survival. While *Coxiella* initially infects alveolar macrophages, in endocarditis patients, it is also found in foamy macrophages containing neutral lipid storage organelles called lipid droplets (LDs). Our previous studies show that *Coxiella* manipulates host LD metabolism via the Type 4 Secretion System (T4SS), a major virulence factor which secretes bacterial effector proteins into the host cell cytoplasm to manipulate cellular processes. Additionally, inhibiting LD breakdown almost completely inhibits bacterial growth suggesting that LD-derived lipids are critical for *Coxiella* intracellular survival. LD breakdown releases arachidonic acid, a prostaglandin E2 (PGE2) precursor, which promotes immunosuppressive environment in alveolar macrophages. Hence we hypothesize that *Coxiella* manipulates host cell LD metabolism to promote a PGE2-mediated immunosuppressive environment and survive long-term in the host. To test this, we quantified PGE2 synthesis enzyme cyclooxygenase-2 (cox-2) gene expression in differentially infected alveolar macrophages. Compared to uninfected cells, cox-2 was upregulated in *Coxiella*-infected macrophages but not T4SS mutant-infected cells. ELISA further showed *Coxiella* infection-dependent increase in PGE2 levels. These studies indicate that during infection *Coxiella* T4SS actively manipulates cox-2 expression resulting in increased PGE2. Studies are ongoing to identify the direct correlation between LDs and PGE2 production in *Coxiella*-infected cells. Future studies will determine the potential of blocking PGE2 production as a supplemental therapy for *Coxiella* endocarditis.

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Type IV Secretion-dependent Inhibition of NF- κ B Transcriptional Networks by *Coxiella burnetii*

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Abstract

Coxiella burnetii is a Gram negative, obligate intracellular pathogen and the etiological agent of Q fever. The organism requires a type IVB secretion system (T4SS) to promote intracellular survival, replication and virulence. Primary murine bone marrow-derived (BMDM) macrophages infected with *C. burnetii* show a remarkable lack of proinflammatory immune responses, even when challenged with the attenuated Nine Mile Phase II (NMII) strain, which is known to stimulate TLR2 through surface-exposed lipoproteins. We hypothesized that the modest host response observed during infection is dependent on the activity of the *Coxiella* T4SS, which may deploy effectors that inhibit innate immune signaling. To identify innate immune signaling pathways that are targeted by the *C. burnetii* T4SS, we used RNA sequencing analysis to compare the transcriptional response of BMDM infected with NMII wildtype, and a *Himar* transposon mutant with an insertion in the *dotA* gene at 8 and 24 hours. We found that between 8 and 24 hours, a total of 216 host transcripts were differentially expressed in a T4SS-dependent manner, with 90 transcripts that were upregulated, and 126 that were downregulated. Causal networks analysis performed using Ingenuity Pathway Analysis software (Qiagen) predicted that the majority of T4SS-dependent host transcriptional regulation resulted from an inhibition of NF- κ B-dependent signaling. Furthermore, we have evidence that at least one T4SS effector expressed by *C. burnetii* contributes to the inhibition of NF- κ B-dependent transcription in infected monocytes. Preliminary data suggest that the activity of this effector is required for the replication of *Coxiella* within BMDM.

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Detection of zoonotic bacterial pathogens in various hosts in the Mnisi community, Mpumalanga, South Africa using a microbiome sequencing approach

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Abstract

The Mnisi community, adjacent to the Kruger National Park, Mpumalanga Province, South Africa, is classified as one of South Africa's 14 rural poverty nodes. It is nestled at the cusp of a human-livestock-wildlife interface. In this area, undifferentiated non-malarial acute febrile illness (AFI) is among the most common presenting signs in patients seeking healthcare at community clinics. Recent research suggested that zoonotic pathogens, either rodent-borne or tick-borne, may be common aetiologies of febrile illness in this community. The aim of this study was to investigate wild rodents, domestic dogs and cattle as possible sources of zoonoses using a microbiome sequencing approach. The bacterial blood microbiome of nine AFI patients, 25 *Mastomys* rodent species, ten dogs and nine cattle were generated using 16S rRNA gene circular consensus sequencing performed on the Pacific Biosciences platform. The rodent microbiome was dominated by *Bartonella* spp. (64%), while *Anaplasma* spp. dominated the dog (36%) and cattle (96.8%) microbiomes. The AFI patient blood microbiome was dominated *Rickettsia africae* (16%), as well as the opportunistic pathogens *Herbaspirillum huttiense* (27%) and *Stenotrophomonas maltophilia* (15.1%). Also noteworthy was the detection of *Brucella melitensis* from one AFI patient. A few sequence reads corresponded to *Coxiella burnetii* (one rodent), *A. phagocytophilum* (one rodent, five dogs and two cattle) and *Borrelia* sp. (one cow). In conclusion, the detection of these zoonotic bacterial pathogens from AFI patient samples, rodents, dogs and cattle highlights their significance as potential contributing factors to non-malarial febrile illness in the Mnisi community area.

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Humoral immunity mediated by human monoclonal antibodies, protects against *Ehrlichia chaffeensis* through novel intracellular mechanisms

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Abstract

Ehrlichia chaffeensis is a zoonotic, gram-negative, obligately intracellular bacterium and the causative agent of human monocytotropic ehrlichiosis (HME). Antibodies are essential for immunity against *Ehrlichia chaffeensis* infection, and the known protective mechanisms involve blocking of ehrlichial attachment or complement and Fc γ -receptor-dependent destruction. In the present study, we determined that human monoclonal antibodies (huMAbs) specific to the major outer membrane protein-1 (OMP-1) hypervariable region 1 (HVR1), are protective through conventional extracellular neutralization, and more significantly through a novel intracellular mechanism. One of the OMP-1-specific huMAb4 engaged intracellular cytosolic Fc receptor, TRIM21 and initiated an innate immune response within minutes, along with rapid intracellular degradation of ehrlichiae. HuMAb4-TRIM21-mediated inhibition was significantly impaired in TRIM21-knockout THP-1 cells. Interaction of huMAb4 coated ehrlichiae with TRIM21 was observed by confocal microscopy and confirmed by co-immunoprecipitation. The *E. chaffeensis*-HuMAb4-TRIM21 complexes induced significant ubiquitination of TRIM21 leading to nuclear translocation of NF- κ B and TRIM21, culminating in the upregulation of proinflammatory cytokine/chemokine transcripts and followed by ehrlichial destruction. Colocalization between huMAb4-opsonized ehrlichiae, polyubiquitinated TRIM21, autophagy regulators (ULK1, Beclin 1) and effectors (LC3, p62) and LAMP2 was observed. Moreover, huMAb4-mediated degradation of *E. chaffeensis* was abrogated by the autophagy inhibitor 3-methyladenine. Our results demonstrate that humoral immunity mediated by OMP-1-specific huMAbs inhibit *E. chaffeensis* infection by a novel intracellular mechanism involving TRIM21, mediating a rapid innate immune response and mobilization of core autophagy components, triggering localized selective autophagic degradation of ehrlichiae.

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Macrophage heterogeneity correlates with the pathogenesis of ehrlichiosis.

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Abstract

Macrophages consist of two well-defined subsets. M1 pro-inflammatory cells that contribute to pathogen clearance, but can also promote tissue injury and M2 anti-inflammatory cells with regenerative roles as they contribute to resolution of injury. Immunocompetent mice infected with highly virulent *Ixodes ovatus Ehrlichia* (IOE) die from systemic inflammation that leads to hepatic damage and organ failure. We examined the heterogeneity and function of the myeloid cell compartments in the liver of mice infected with IOE and mildly virulent *Ehrlichia muris* (EM) strains; murine models of fatal and mild ehrlichiosis, respectively. We found that livers from IOE-infected mice showed higher frequencies and accumulation of infiltrating F4/80^{lo}CD11b^{hi} monocytes expressing iNOS and granzyme B compared to EM infection (9 fold increase). M1 polarization in the liver of IOE-infected mice coincide with induction of pathogenic adaptive responses, excessive inflammation, and liver injury. *In vivo* data was also confirmed *in vitro* using bone-marrow-derived macrophages (BMM). Results showed that IOE promoted polarization into M1 macrophages expressing significantly higher levels of MHC-II, iNOS, and TNF- α compared to EM. In contrast, EM, but not IOE, infection induced significant polarization into M2 macrophages expressing CD206, arginase-1, and TGF- β compared to controls. Notably, EM infection induced higher autophagy flux defined as enhanced induction and autophagosome-lysosomal fusion in BMM compared to IOE infection. These data suggest that M1 monocytes may promote liver damage caused by dysregulated innate and adaptive immune responses during fatal ehrlichiosis. Thus, M1 monocyte targeting may represent a therapeutic approach against hepatic pathogenesis induced by fatal *Ehrlichia* infection.

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Thailand-Myanmar mil-mil collaborative surveillance program for rickettsiosis

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Abstract

Thailand and Myanmar established a mil-mil surveillance program aiming to gain information of rickettsial pathogens spreading in the Republic of the Union of Myanmar and Thailand as well as to investigate the transmission cycle. Scrub typhus outbreak investigation in Thailand and Myanmar febrile disease surveillance were performed as jointed working during September-November 2018. Captured rodents in Thailand outbreak areas showed 28% and 6% positive for scrub typhus and murine typhus agents, respectively. 17 kDa rickettsiae gene was detected in 53.3% (16/30) of collected blood-sucking arthropods (6 tick, 9 flea, and 1 louse pools). *Rickettsia asembonensis* were identified in 9/16 of positive arthropods. Of total 200 febrile patients recruited from military health facilities throughout the Republic of the Union of Myanmar, 8% and 0.5% displayed seroconversion to scrub typhus and murine typhus agents, respectively. A total of 62 blood-sucking arthropod pools were collected from pets, poultry, livestock and rodents in Ywar Thit village around Tatfone region, Myanmar. Rickettsial 17 kDa gene was detected in 35.5% of collected arthropods (6/30 tick, 13/23 flea, and 3/8 louse pools). *R. asembonensis* were identified in 17 of 22 *Rickettsia* positive arthropod pools. These mil-mil surveillance activities clearly demonstrated the presence of *Rickettsia* infection in this region. Specific information regarding the causative agents and transmission of rickettsial diseases is essential to establish an effective disease prevention and control program. Experience sharing during these mil-mil activities will also extend the capacity for outbreak response and active surveillance of these arthropod-borne febrile illnesses among ASEAN countries.

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Disruption of ORP1L (Oxysterol Binding Protein 1 Long) in murine alveolar macrophages attenuates *Coxiella burnetii* intracellular growth

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Abstract

Coxiella burnetii is an intracellular bacterium that causes human Q fever. *Coxiella* infects humans through infectious aerosols, and alveolar macrophages are the primary target of infection. Within host cells, *Coxiella* thrives within a specialized parasitophorous vacuole (PV) that is essential for *Coxiella* pathogenesis. The PV membrane is rich in sterol species, and cholesterol was previously thought to be beneficial to *Coxiella*. However, our lab has established that cholesterol is not required for *Coxiella* growth, and that elevated PV cholesterol is toxic to the bacteria. A major focus of our research has been investigating *Coxiella*'s ability to mitigate the detrimental effects of cholesterol during infection. Previous work demonstrated that the host cholesterol-binding protein ORP1L localizes to the *Coxiella* PV. ORP1L mediates trafficking of late endosomes and lysosomes and transports cholesterol from these organelles to the endoplasmic reticulum. ORP1L's cholesterol transport function and early localization to the PV lead us to hypothesize that ORP1L may be important for *Coxiella*'s survival strategy. We generated ORP1L-knockout murine alveolar macrophages (MH-S cells) using a CRISPR-Cas9 system, and performed CFU growth assays to assess *Coxiella* growth in knockout cells. Bacterial growth was significantly decreased (2-5 fold, n=4) in two knockout clones compared to wildtype MH-S cells. Additionally, PV size was significantly smaller by immunofluorescence in the knockout clones six days post infection. Ongoing work will determine the specific function of ORP1L at the *Coxiella* PV membrane.

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***Orientia tsutsugamushi* modulates cellular levels of NF- κ B p105**

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Abstract

The transcription factor NF- κ B, a key initiator of the antimicrobial response, is tightly regulated. I κ B α and p105 bind to and sequester NF- κ B in the cytoplasm. Canonical activation of NF- κ B involves IKK-mediated phosphorylation of I κ B α and p105, which leads to their ubiquitination and proteasomal degradation and hence induces NF- κ B nuclear translocation. A portion of ubiquitinated p105 is processed into NF- κ B p50. *Orientia tsutsugamushi* causes the life-threatening disease, scrub typhus. We recently reported that *O. tsutsugamushi* inhibits NF- κ B nuclear accumulation using two effectors, Ank1 and Ank6, that act downstream of I κ B α degradation. Whether the pathogen targets other portions of the canonical NF- κ B pathway is unknown. Here, we demonstrate that p105 levels are significantly elevated in *O. tsutsugamushi* infected HeLa cells at 24 h and continue to increase to 72 h where they are approximately six-fold higher versus uninfected cells. The *O. tsutsugamushi*-stimulated increase in p105 is bacterial dose-dependent. Notably, p50 levels are increased only 1.7-fold during infection. A similar trend was observed for infected RF/6A endothelial cells. Whereas NF- κ B nuclear accumulation was blocked in HeLa cells ectopically expressing Ank1 or Ank6, p105 levels were unaffected. Experiments are underway to determine if these phenomena are protein synthesis-dependent and extend to p105 transcriptional expression. *O. tsutsugamushi* therefore promotes upregulated expression and/or inhibits proteolytic processing of p105 in an Ank1- and Ank6-independent manner, which likely contributes to its negative regulation of the canonical NF- κ B pathway. Collectively, these data and our prior report demonstrate that *O. tsutsugamushi* utilizes a multi-pronged approach to antagonize NF- κ B.

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Identification of small RNAs expressed *in vitro* by *Bartonella bacilliformis* under a variety of conditions that simulate the human host and sand fly vector

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Abstract

Bartonella bacilliformis is a Gram-negative, facultative intracellular alphaproteobacterium and zoonotic agent of Carrión's disease. Carrión's disease is an emerging but neglected tropical disease endemic to Peru, Colombia and Ecuador. The illness is often biphasic, with the first stage manifesting as an acute hemolytic anemia and the second stage presenting with blood-filled hemangiomas of the skin. *B. bacilliformis* is spread between humans through the bite of phlebotomine sand flies. As a result, the pathogen encounters significant environmental shifts during its lifecycle, including changes in pH and temperature. In most bacteria, small non-coding RNAs (sRNAs) serve as regulatory molecules involved in the control of nearly all biological processes. However, no sRNAs have been identified in *Bartonella*, to date. We therefore performed small RNA-Seq analyses on *B. bacilliformis* grown *in vitro* under various temperature (solid media) and pH (liquid media) shifts designed to simulate conditions encountered by the pathogen during its lifecycle. As a result, we discovered roughly 84 putative sRNAs. Fourteen of the sRNAs were uniquely expressed during pH-shifts, whereas sixty were unique to temperature-shifts. Identified sRNAs include the widespread, highly conserved tmRNA and 6S RNA, the conserved alphaproteobacterial sRNAs ar45 and speF, a novel group I intron unique to *Bartonella* spp., plus 79 other sRNAs of unknown function. Northern blots have confirmed the expression of eight sRNAs, to date. We are currently examining splicing of the group I intron as well as the function of a highly-expressed "pH-specific" sRNA predicted to regulate transcripts for various outer membrane proteins of the pathogen.

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***Coxiella burnetii* infections in mice: Clinical and immunological responses to contemporary genotypes**

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Abstract

Today, three genotypes of *Coxiella burnetii* are found in the US, and have been correlated with specific animal hosts. The ST20 genotype is unique to cows, is found in commercial milk products, but is rarely implicated in human infections. The ST8 genotype is found in goats, has been linked to outbreaks, and is frequently associated with human chronic infections. The ST16 genotype lacks a strong linkage to specific hosts, and is not typically implicated in human infections. In this study, groups of mice were infected via aerosol inoculation with ST20, ST8, and ST16 isolates derived from environmental, animal, and human samples. Mice were monitored for illness after infection, and routine blood draws were performed during the study. Animals were euthanized at 2 and 12 weeks post-infection, and bacterial burden was determined for multiple tissue types by PCR. Serum samples were tested by IFA in order to determine the serological response over time. Weight loss and splenomegaly were observed in mice infected with ST20 and ST16 isolates, but were absent in the mice infected with ST8 isolates. Bacterial concentrations in the tissues were lower in the ST8 isolates relative to all other isolates. ST16 and ST20 isolates produced a robust immune response, while ST8 isolates initially produced significantly lower titers then saw increased titers in some animals several weeks post-infection. The data suggest that the ST8 isolates produce weak antibody responses in mice that are slow to develop. This may allow low levels of *C. burnetii* to persist after ST8 infections.

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Investigation of a Novel Uncharacterized Rickettsiales in *R. amblyommatis* isolate Ac37

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Abstract

Rickettsia amblyommatis is found in several *Amblyomma* spp. ticks in the Americas. We examined several *R. amblyommatis* strains from North and South America for the presence of plasmids and sequenced their genomes in order to better understand differences between the strains. Genomic sequencing of strain Ac37 originally isolated from *Amblyomma* ticks collected in Brazil (Labruna et al. 2004 J Med Entomol 41:1073-81) revealed the presence of a second Rickettsiales species in isolate Ac37. Genomic analysis indicated that the unknown Rickettsiales contained 2 plasmids. Efforts to isolate the Rickettsiales alone in ISE6 cells were unsuccessful and transmission electron microscopy studies to identify it were inconclusive. Shuttle vectors using Spectinomycin or Rifampicin selection with GFPuv, Cherry or Far Red reporter genes were constructed based on sequences from one of the plasmids and used to transform isolate Ac37. It was hypothesized that electroporating isolate Ac37 with a shuttle vector specific for the new Rickettsiales species under antibiotic selection would select for transformants of the new Rickettsiales and eliminate *R. amblyommatis*. PCR primers specific for each of the 2 organisms were used to screen Ac37 transformants for the presence or absence of each organism. None of the shuttle vectors allowed us to select for the new Rickettsiales by eliminating *R. amblyommatis* from isolate Ac37. Transformation of Ac37 with the rickettsial shuttle vector pRAM18dRGA[MCS] eliminated the new Rickettsiales yielding a culture containing only *R. amblyommatis*. The new Rickettsiales based shuttle vectors failed to transform other rickettsial species.

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Anaplasma marginale outer membrane protein vaccine candidates are conserved in North American and South African strains

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Abstract

Bovine anaplasmosis is a globally economically important tick-borne disease caused by the obligate intraerythrocytic rickettsia, *Anaplasma marginale*. A live *Anaplasma centrale* blood-based vaccine is available, but does not protect against all *A. marginale* field strains and may also transmit other blood-borne pathogens. Five potential outer membrane protein (OMP) vaccine candidates have been identified and well-characterised in *A. marginale* strains from the USA. We evaluated the presence and genetic diversity of *A. marginale* in South Africa, and characterized the five OMP vaccine candidates in *A. marginale*-positive samples. *Anaplasma marginale* and *A. centrale* were detected using a duplex quantitative real-time PCR in 57% and 17%, respectively, of 517 bovine blood samples, with 15% being co-infected. South African strains were suggested to be highly diverse by *msp1a* genotyping. OMP genes *Am779*, *Am854*, *omp7*, *omp8* and *omp9* were amplified and sequenced from South African samples with differing *msp1a*-genotypes. OMPs Am854 and Am779 were highly conserved, with 99–100% amino acid identity with US strains, while Omp7, Omp8 and Omp9 had 79–100% identity. As shown previously, Omp7–9 possess conserved N- and C- termini, a central variable region, and a highly conserved CD4 T-cell epitope in the N-terminal region. Western analysis of recombinant OMPs using sera from animals immunized with US OMP preps indicates strong antigenic conservation between South African and US strains of *A. marginale*, suggesting that they are good candidates for use in a novel global vaccine cocktail, although further work on the best formulation and delivery methods will be necessary.

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Development of Highly Sensitive and Specific Acute Diagnostic Tests for Tick-Borne Spotted Fever Rickettsioses

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Abstract

Tick-borne rickettsial diseases continue to cause severe illness and death in otherwise healthy individuals, with pre-antibiotic case-fatality rates reported as high as 65%-80% in some case series. Despite the availability of low-cost, effective antibiotic treatment, spotted fever rickettsioses still cause significant morbidity and mortality because of the unavailability of acute laboratory diagnostic tests. Using an affinity enrichment-mass spectrometry method (IP-MS) and immunoblotting, we have identified RC0497 in the supernatant of *R. conorii*-infected primary human umbilical vein endothelial cells (HUVECs). A panel of mouse monoclonal and rabbit polyclonal antibodies against RC0497 have been generated and tested for their binding affinity to RC0497. By using these antibodies, we were able to detect as a minimal concentration as 0.15 µg/ml of RC0497 spiked in human sera in a sandwich enzyme-linked immunosorbent assay (ELISA). Strikingly, tryptic peptides of RC0497 were also detected by IP-MS in the circulating sera of *R. conorii*-infected mice at levels differentiating sublethal from lethal infections at the early stage of infections. Immuno-detection of RC0497 in the acute sera of patients and experimentally infected animals by ELISA is currently under investigation. Taken together, these studies have paved the way for developing a novel, highly sensitive, and specific diagnostic assay with prognostic value for diseases caused by *Rickettsia*.

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Serological and clinical features of patients with PCR-proven infection by *Anaplasma phagocytophilum*

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Abstract

Anaplasma phagocytophilum infection (human granulocytic anaplasmosis [HGA]) diagnosis is potentially established by PCR, 4-fold antibody titer increase, culture, and/or blood smear. To better characterize HGA serologic reactions, we reviewed clinical, laboratory, and serological results in PCR-proven patients. Using a serum repository from patients with suspicion for HGA between 1992 and 2017, we evaluated 74 PCR+ patients. Serodiagnosis was defined as a 4-fold titer increase between acute and convalescent sera with a minimum ³160, or single acute phase ³320. Fifty-eight PCR+ patients had 4-fold convalescent titer increases and 13 had single acute phase titers >320 at presentation; 2 had serologic reactions that did not fulfill serodiagnosis definition when tested at d37 and d182; 1 did not develop a serologic reaction at d22. Sera were available up to 1,853d after the first sample. 2-16 samples were tested per patient; 26 of 98 (27%) at d0 had a titer >80; 75% and 100% were positive at 8-14d and 15-21d, respectively. Median IFA titer peaked at 1,280 (IQR 320-2560) at 46-90d. All PCR+ patients had similar clinical findings except for weakness (14% with vs. 42% without serodiagnosis [p=0.010]). A titer of ³80 was found in >33% of PCR-confirmed patients at >2 years after diagnosis (median 40 [max 2560]), and could still be detected in 5/19 (26%) after 3 years (range 160-2560). Single serum testing, even with a titer ≥2560, should not be considered as diagnostic of active or recent HGA absent consistent clinical findings and a positive direct test such as PCR.

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The analysis of plasma metabolites in mice survived against the *Orientia tsutsugamushi* infection

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Abstract

Tsutsugamushi diseases, caused by *Orientia tsutsugamushi* infection, have been characterized by an acute, febrile, and potentially fatal mite-borne one. Until now, many studies a tsutsugamushi disease has been predominantly focused on the early infection mechanisms, but it's still necessary to understand the host recovery mechanisms in a tsutsugamushi disease. To do this, in this study, balb/c mice were infected by *O. tsutsugamushi* and metabolic profiles in the plasma from dead and recovered mice after lethal dose 50 (LD50) treatment of *O. tsutsugamushi*. We analyzed the plasma using liquid chromatography-(LC-) and flow injection analysis-tandem mass spectrometry (FIA-MS/MS). As a result, 87 metabolites were identified, whose concentration significantly changes in the plasma from experimental groups. Major metabolite classes were acylcarnitines, glycerophospholipids, biogenic amines and amino acids. Among them, 83 metabolites were reduced and 4 metabolites were increased in plasma from the dead mouse group. However, 84 metabolites were increased and 3 metabolites were decreased in plasma from recovered mice, when compared to dead mice. Major perturbed metabolic pathways included tryptophan, glycerolipid and phospholipid metabolism, which suggests the multi-factorial nature of host responses. This finding provided an important platform for further investigation of the scrub typhus recovery mechanisms associated with *O. tsutsugamushi* infection. This study was supported by a research grant (2018-NI002-01) of Korea Centers for Disease Control and Prevention.

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Surveillance on Rickettsial Disease in the country of Georgia

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Abstract

Rickettsioses are a group of diseases caused by species of *Rickettsia*, a genus of obligate intracellular bacteria. Most of the rickettsioses are transmitted by ticks, but they can also be transmitted by fleas, lice and mites. One species, *R. prowazekii*, is categorized by the US CDC as a Category B Select Agent due to past efforts to weaponize the bacteria. Thus, identification and knowledge of the circulating rickettsial pathogens are critically important to determine the risk and management of serious rickettsial disease. Georgia is located in the Caucasus region, where it is bordered by Azerbaijan, Armenia, Russia, and Turkey. An overview of existing published data was conducted to assess the situation regarding rickettsial diseases in the Caucasus region. In a study, conducted in Georgia in 2008, it was concluded that 7% of cases of acute undifferentiated fever were seropositive for antibodies against typhus group *Rickettsia*. In another study, 32.5% of the obtained tick pools were positive for *Rickettsia* spp., in 2015. In Turkey, 10.5% of tick samples were positive for *Rickettsia* spp. That were collected from humans. In Armenia, only one case of rickettsiosis was found in 2014. No data has shown existence of *Rickettsia* in Azerbaijan. Data has shown that *Rickettsia* is present in the Caucasus region. It should be suggested that the burden of rickettsial diseases are not understood well enough in the region, and more thorough investigations should be conducted by using One Health approach.

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Requests for rickettsial disease diagnostics over 6 years in Sri Lanka; room for improvement?

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Abstract

Rickettsial Disease Diagnosis and Research Laboratory (RDDRL) was established in the Faculty of Medicine, University of Kelaniya, Sri Lanka in August 2008 with view to improve management of the illness. IFA-IgG titre against *Orientia tsutsugamushi*(OT) or *Rickettsia conorii*(RC) antigens >1:128 in an acute sample was considered positive for scrub typhus(ST) or spotted fever group rickettsioses(SFGR) respectively. All government hospitals were informed of the availability of service free of charge. Presentations and publications were carried out to enhance knowledge of illness, with emphasis on early diagnosis and treatment to prevent serious complications. RDDRL database was analyzed to assess the improvement in clinical care by comparing the illness duration (time delay) when diagnostics were requested (IDDR), between the years 2011 and 2016. Of 3017 samples received, 558(18.5%) confirmed positive for acute rickettsioses; ST 43%, SFGR 51.3% and 5.7% for both. Highest confirmed districts were Gampaha, Colombo, Kurunegala and Puttalam. In 2011, IDDR: Colombo; 14.13days (SD:5.66) compared to 12.5days (SD:11.14) in 2016. In Gampaha: 2011; 12.3days (SD:6.21) compared to 10.38days (SD:5.23) in 2016 (p<0.05). The requests for confirmed ST in Colombo: 2011; 15.4 days(SD:7.2) and 2016; 13.7days(SD:13) compared to Gampaha: 2011; 14.2days (SD:6.7) and 2016; 9.6days (SD:4.3)(p<0.05). That for SFGR in Colombo: 2011; 13.5days (SD:4.2) and 2016; 12days (SD:8), and Gampaha: 2011; 11.1days (SD :5.3), 2016: 10.8days (SD:5.6). Although a considerable improvement is noted in IDDR with significant improvement where RDDRL is located, it remains more than a week for both infections.

Evidence of exposure to spotted fever group rickettsioses (SFGR) and *Orientia tsutsugamushi* (OT) in Gampaha and Colombo districts in Western Sri Lanka

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Abstract

Tick-borne SFGR, caused by *Rickettsia conorii* (RC) and mite-borne scrub typhus (ST) caused by OT are re-emerging in the endemic Wet Zone of Western Sri Lanka (WZWSL). The rates of exposure for SFGR and ST in predefined age groups, their gender and occupations were studied among a cohort of febrile hospitalized patients admitted during 2011-2016 in Colombo (urban) and Gampaha (semi-urban) districts in WZWSL. The study cohort included patients with a rickettsia IFA-IgG titre of $\geq 1:32$ and $\leq 1:128$ against RC or OT antigens with no acute rickettsial illness (indicating exposure) and IFA-IgG negatives (no exposure). 1146 individuals were selected; Colombo: 473 (63% males); [Mean age 36.6 yrs (SD 17.8)] and Gampaha 673 (59% males); [Mean age 35.1 yrs (SD 18.9)]. In Colombo and Gampaha, exposure to SFG/OT was 235 (49.7%) and 318 (47.3%) respectively. They included 51.8% of males & 48.4% of females in Colombo and 48.4% of males and 45.6% of females in Gampaha. Exposure; SFG:OT in Colombo; 169 (35.7%): 106 (22.4%) and in Gampaha 234 (34.8%): 160 (23.8%). For both districts, exposure rates among age groups 0-5yrs(pre-school); 6-19yrs (school), 20-55yrs (employed) and >55years were 31.9%, 32%, 32.2% and 32.8% respectively and were high among retired males (64%), masons (57%), students (46%) and housewives (44%). In both urban and semi urban wet zone of Western Sri Lanka, exposure to SFG was more than OT, with all age groups having similar exposure and retired males, masons, housewives and students had the highest exposure rates probably suggesting a peri-domestic acquisition.

**30th Meeting of the
American Society for Rickettsiology:
Rickettsial Diseases at the
Vector-Pathogen Interface**



**June 8-11, 2019
El Dorado Hotel,
Santa Fe, New Mexico**



Monday, June 10, 2019
Abstracts

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.

Session 5: Ecology and Epidemiology

Chair: Kevin Macaluso & Maria Galletti

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Flea-borne rickettsial disease outbreaks: what are the causative agents?

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Abstract

Flea-borne rickettsiae include *Rickettsia typhi* the causative agent of murine typhus (aka endemic typhus or flea-borne typhus), *Rickettsia felis*, the causative agent of flea-borne spotted fever, *Rickettsia asembonensis*, reported to be associated with human infections, *Candidatus Rickettsia senegalensis*, an incompletely characterized rickettsial agent with unknown pathogenicity. These agents and the diseases they caused are found throughout the world due to the distribution of their flea hosts that include the Oriental rat flea (*Xenopsylla cheopis*) for *R. typhi*, and the cat and dog fleas (*Ctenocephalides felis* and *Ctenocephalides canis*, respectively) for *R. felis*, *R. asembonensis* and *Ca. R. senegalensis*. Murine typhus, is found in endemic foci, especially in urban settings around the world. Flea-borne spotted fever has been associated worldwide with sporadic illnesses in urban, suburban and rural areas and most commonly in sub Saharan Africa. Recent reports of human infection with *R. asembonensis* have been reported from Malaysia, Thailand and Peru. The current presentation of these agents in the same locations has muddied the waters in determining the cause of flea-borne rickettsial diseases, such as finding evidence of infection with *R. typhi* in cat fleas and opossums in suburban settings where *R. felis* is more commonly found. This presentation describes the current situation of flea-borne rickettsial diseases and methods to clear the waters in determining the cause or causes of flea-borne rickettsiosis outbreaks.

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Tick-borne encephalitis virus and *Rickettsia* spp. in ticks collected from birds in Hesse, Germany

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Abstract

Tick-borne diseases are being recognized as a growing public health concern and cause significant health issues in humans and animals. Birds, especially migratory birds, contribute to the spread of ticks and tick-borne diseases. Only a few studies have been carried out in Germany so far to investigate the tick fauna on birds and pathogens they might harbor. We have captured a total of 10,286 birds in a northern region of the Federal State of Hesse, Germany, in 2017 and 2018, from which 450 were infested with ticks. Most of the ticks were *Ixodes ricinus* (95.4%) nymphs and larvae. However, some ornithophilic species were identified: *I. frontalis* (larvae, nymphs and females) and *I. arboricola* (larvae and nymphs). Ticks were tested individually or in pools, depending on the tick life stage and species for each bird host, to detect tick-borne encephalitis virus and *Rickettsia* spp. All the samples tested negative for tick-borne encephalitis virus. Of 485 investigated tick pools, 162 were tested positive for *Rickettsia* spp. by a screening PCR. Of these, 117 showed positive results in a *Rickettsia helvetica* specific real-time PCR. Samples which were negative in the *R. helvetica* PCR were further investigated by five targets using multilocus sequence typing. The obtained sequences were identified as a *Rickettsia* species with the closest relation to the validated species *R. japonica* and *R. heilongjiangensis*, respectively in *I. arboricola* and three as *R. helvetica* in *I. ricinus* and *I. frontalis*.

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The coinfection with *Ehrlichia minasensis*, *Anaplasma marginale* and a new *Anaplasma* genotype is not associated with anemia in beef cattle in the Brazilian Pantanal, an endemic area for bovine trypanosomiasis in South America

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Abstract

The present study aimed at investigating the occurrence and genetic diversity of *Anaplasma* and *Ehrlichia* in beef cattle (*Bos indicus*) sampled in the Brazilian Pantanal, an area prone to periodic flooding and endemic for bovine trypanosomiasis. Blood samples from 400 cattle were collected and submitted to quantitative real-time PCR and conventional PCR assays for *Anaplasma* spp. based on *msp1β*, *msp-2*, *msp1α* and *rrs* genes, and PCR for *Ehrlichia* spp. based on *dsb* gene. The endemicity of the area for *Trypanosoma vivax* was confirmed by indirect ELISA (89.75% seropositivity). The qPCR (*msp1β*) revealed 56.75% positivity for *A. marginale*. The analysis of the obtained *A. marginale msp1α* showed the presence of E (76.9 %), C (15.38%) and B (7.69%) genotypes. Fourteen *A. marginale* strains were detected, with eight newly described (τ-10-13-13-18; τ-27-18; EV8-EV8-17; α-β-β -β-100; EV7-11-10-15; τ-11-11-27-18; τ-11-10-15; τ-27-13-18). *Anaplasma* spp. *rrs* phylogenetically associated with *A. phagocytophilum*, but negative on the qPCR specific based on the *msp-2* gene, was detected in 4.75% animals. Finally, 41.75% animals were positive for *E. minasensis*. Regarding coinfection, 2.5% animals were positive for the three agents simultaneously; 3% for *A. phagocytophilum*-like and *E. minasensis*; 4% for *A. phagocytophilum*-like and *A. marginale*; 26.5% were positive for *A. marginale* and *Ehrlichia minasensis*. All animals showed hematocrit greater than 24%, except for one cow, which was negative for the studied agents. It is concluded that beef cattle in the Brazilian Pantanal are exposed to infection by a great diversity of Anaplasmataceae agents, which apparently is not associated with anemia.

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Ehrlichiosis in Adair County, Missouri from 2014 to Present

Deborah Hudman

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Abstract

In 2014-15, four conservation areas were surveyed for the presence/absence of human pathogens in ticks. *Ehrlichia chaffeensis* and *Ehrlichia ewingii* were present in *Amblyomma americanum* (Aa) and *Dermacentor variabilis* (Dv). Infection rates for *E. chaffeensis* were 19% for both species of adult ticks (Aa n=436; Dv n=189) and *E. ewingii* infection rates were 3% for Aa and 15% for Dv. With *Ehrlichia* quantified in local ticks, the prevalence of residents acquiring tick-borne diseases needed investigation. In 2018, surveys revealed that 96% of respondents reported finding attached ticks on their person; 17% are removing 11 or more attached ticks per year; 38% developed symptoms post tick bite. In addition, 89% of practitioners had treated at least one patient for tick-borne diseases and 44% are treating 11 or more patients per year. Understanding how the environment relates to spread of disease is essential for effective management of disease risks. Of the four conservation areas surveyed in 2014-15, two areas were actively managed while two were unmanaged. The number of ticks was nearly double in the unmanaged sites. Research focus is now on management of tick populations through prescribed burns. Beginning in 2018, a 1500-acre, oak-hickory forest divided into twelve units was surveyed for ticks and their pathogens. Over a three year period, nine units will be burned in rotation while three will not be burned. Thus far, the prevalence of tick-borne pathogens is more or less similar between sites, but tick numbers are dramatically lower in sites burned since 2016.

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Flea-borne *Rickettsia* in Pennsylvania

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Abstract

Flea-borne spotted fever, caused by *Rickettsia felis*, is transmitted to humans by the cat flea (*Ctenocephalides felis*). Due to the ubiquitous geographic distribution of the cat flea, *R. felis* is an emergent threat to humans worldwide. *Rickettsia asembonensis* and *R. felis* like organisms (RFLOs) have also been detected in fleas; their pathogenicity is unclear. Recent studies demonstrated a link between opossums/feral cats, and their role in the zoonotic spread of vector-borne agents. Pennsylvania harbors a large population of feral cats, opossums, and wild canids. Fleas were collected from feral cats at Trap/Neuter/Release events and from opossums that were trapped/combed (southcentral Pennsylvania); and from hunter-killed wild canids (statewide). 407 fleas were collected; *C. felis* was found on 24/100 cats (36/135 (26.7% positive) for *Rickettsia* 17-kDa antigen gene (Rick17b qPCR assay)). *C. felis* was found on 8/49 opossums (42/96 (43.8%) positive for Rick17b). Other fleas found on opossums (11/49) were *Nosopsyllus fasciatus* (3/56 (5.4%) positive) and one *Cediopsylla simplex* (negative) by Rick17b. Wild canids harbored: *C. felis*, *Ctenocephalides canis*, *C. simplex*, *Leptopsylla segnis*, and *Orchopeas howardii*. Fleas positive for Rick17b were *L. segnis* 2/14 (14.3%), *C. felis* 1/1 (coyotes); *C. simplex* 6/13 (46.2%), *C. canis* 1/1, *L. segnis* 1/10 (10%) (red foxes); and *L. segnis* 10/39 (25.6%) (gray foxes). Fleas are being analyzed using *Rickettsia* species-specific qPCR assays and MLST. Blood samples from the animals are being analyzed using qPCR and ELISA. Due to the suburban dwelling of feral cats/PA wildlife, residents/domestic pets could potentially be exposed to flea-borne rickettsial diseases.

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A Preliminary Plan For Instituting A State-Wide Tick Management Program

Lauren Maestas

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Abstract

Tick management is a difficult subject to broach. Unlike mosquito control, which has been implemented in the United States since about 1903, tick control is a relatively new concept. Complex aspects of tick ecology complicate the development of control methods. Various avenues of tick control have been pursued but are often unsuccessful when used individually. This has resulted in a push for integrated tick management in a one-health framework has resulted. Few programs exist with a goal of widespread tick management. Those that do are usually small-scale, targeted operations focused on one aspect of control. As tick-borne diseases are increasingly recognized, the need for effective tick management strategies becomes more apparent. This presentation is intended to serve as a catalyst for conversation on implementation of a statewide tick management program. I will talk about the preliminary steps currently being taken in Delaware with the goal of establishing a statewide tick management program. I will talk about recent literature and how it applies to tick management, offering suggestions for developing a framework for a large scale, long-term, and sustainable integrated tick management program. I will touch on aspects of passive and active surveillance, and the avenues we are pursuing. Finally I will tell you about our future plan to institute chemical and mechanical control in an economically sustainable manner.

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The Case for Passive Surveillance: Tick Testing and Military Populations

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Abstract

Since 1989, the Army Public Health Center Tick-Borne Disease Laboratory (TBDL) has operated the Department of Defense Human Tick Test Kit Program (HTTKP), which receives human-biting ticks removed from military-affiliated personnel. These ticks are identified, assayed for human pathogenic agents, and the results are reported back to the tick bite victims and their physicians as actionable evidence for use in determining the appropriate treatment regimen. The TBDL has amassed large, long-term datasets from many of the installations that submit ticks to the program. This enables the TBDL to identify trends in tick and pathogen presence across a broad geographic scale, and to provide up-to-date surveillance data and recommendations targeted to the specific risks present at each installation. The TBDL also offers information and educational materials regarding ticks, tick-borne diseases, tick removal, and personal protective techniques. Here we describe highlights from HTTKP surveillance, and describe findings from some other collaborations undertaken by the TBDL. We will also discuss the CDC recommendations against the use of tick testing programs, and will provide evidence for the use of these services under certain circumstances, with a focus on military populations.

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Confirmation of *Anaplasma phagocytophilum* in South Africa by multilocus sequencing

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Abstract

Although *Anaplasma phagocytophilum* (Ap) has been reported across the breadth of Africa, these reports generally have relied on detection of DNA of a single gene. Further, in recent years, there has been an increasing number of distinct *Anaplasma*-like 16S rRNA gene sequences deposited in the databases, with little characterization of the agents that these sequences are derived from. These uncharacterized *Anaplasma*-like agents may contribute to cross reaction in molecular tests. Recent studies have reported a new *Anaplasma* agent referred to as *Anaplasma* sp. ZAM dog or *Anaplasma* sp. SA dog (Adog). In the present study we analyzed samples from an array of hosts, including humans, rodents, cattle, dogs and ticks. A previously reported assay based on the *msp2* gene of Ap yielded positive results with all sample sets (10-85%), however the test was found to cross-react with Adog. Select samples positive with this assay were analyzed for 16S rRNA, *gltA*, *msp4* and *AnkA*. Sequencing and phylogenetic analyses of these genes showed that the 16S rRNA gene and *gltA* were useful to discriminate Ap from Adog, while *msp4* and *AnkA* were identical to Ap, but because there are no known sequences for these genes from Adog, we could not definitively say they were only from Ap. Our data show the detection of Ap in dogs, rodents and humans with acute febrile illness (AFI). We also detected a number of other *Anaplasma* species that may be zoonotic agents. We recommend that Ap be considered in the differential diagnosis of non-malarial AFI.

Session 6: Vaccines/Infection, Diagnosis & Treatment

Chair: Steve Dumler & Katelynn Doiron

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***Neorickettsia finleia* sp. nov., a bacterium that causes Potomac horse fever**

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Abstract

Potomac horse fever (PHF) is a severe and frequently fatal febrile diarrheal disease caused by *Neorickettsia risticii*. *Neorickettsia* species are endosymbionts of digenetic trematodes. When horses ingest adult insects carrying encysted *N. risticii*-infected trematode metacercariae, the bacterium is transmitted from the trematodes to the horses. Herein, we report the identification and characterization of a new *Neorickettsia* species found in two locations in eastern Ontario, Canada in 2016 and 2017 (in addition to 10 variable strains of *N. risticii*) that causes clinical signs indistinguishable from those of PHF. The nucleotide sequences of the genes encoding for 16S rRNA and the amino acid sequences of the major surface antigen P51 from culture isolates of this new *Neorickettsia* species were distinct from those of all previously characterized *N. risticii* strains and *Neorickettsia* species, except for those from an uncharacterized *Neorickettsia* species culture isolated from a horse with PHF in northern Ohio in 1991. Conversely, the surface antigen Ssa3 amino acid sequence and intramolecular repeats within Ssa3 of the newly identified *Neorickettsia* species were similar to those of *N. risticii* but not to those of other *Neorickettsia* species. Western immunoblot analysis showed antigen profiles of the newly isolated *Neorickettsia* species recognized by PHF sera were distinct from those of *N. risticii* strains. We propose to classify this new bacterium as *Neorickettsia finleia* sp. nov. The finding will improve the laboratory diagnosis and vaccine for PHF, and understanding of the PHF pathogenesis, the geographic prevalence and distribution of diverse *Neorickettsia* species in nature.

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High-throughput screening of modulators of cellular calcium metabolism as potential drugs against rickettsia-induced microvascular dysfunction

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Abstract

Background: Tick-borne rickettsiae cause severe disease in up to >20% of patients, even if treated. The pathophysiology of severe disease is increased microvascular endothelial cell (MEC) barrier permeability. Control of cellular Ca²⁺ metabolism is essential for MEC barrier function. Using in vitro human MEC, we showed spotted fever *Rickettsia* and *Borrelia burgdorferi* caused Ca²⁺-dependent disruption of barrier integrity. Methods: We tested >20 pharmacologic modulators of Ca²⁺ signaling for the ability to prevent MEC dysfunction with *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* and *Rickettsia parkeri*. We used human brain MEC (HBMEC) in 96-well microelectrode arrays with static electric cell impedance sensing (ECIS) to monitor barrier integrity with treatments. Cellular and bacterial toxicity was examined using cell viability and regrowth, respectively. At maximum resistance, HBMEC were pre- or sham-treated with drugs and then with *A. phagocytophilum*-infected HL-60 cells, *E. chaffeensis*-infected THP-1 cells, or cell free *R. parkeri* at 10-50 MOI. Results: Drug concentrations <10 µM or <10 µg/mL were nontoxic for HBMEC. Toxicity for *ex vivo* human neutrophils was observed at (1 µM for 3 drugs. Toxicity against *A. phagocytophilum* was demonstrated at concentrations (1 µM for 3 drugs. Over 72 hours, membrane-active chelators DPb99 and DP460, and the calcium channel blocker benidipine, in a dose-dependent manner, stabilized and prevented pathogen-induced MEC integrity disruption at low, non-toxic drug concentrations. Conclusions: Drugs known to affect Ca²⁺ flux stabilized rickettsia-induced vascular permeability without directly impacting rickettsial viability. These data provide support for host-directed therapeutics for rickettsial disease, and perhaps others that promote vascular permeability.

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***Anaplasma marginale* infection of *Dermacentor andersoni* through an *in vitro* tick feeding system**

Rubikah Vimonish, Wendell Johnson, Glen Scoles, Susan Noh, Massaro Ueti

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Abstract

We developed an *in vitro* tick feeding system to study tick-borne pathogens of veterinary importance. We fed *D. andersoni* adult ticks with blood from an *A. marginale*-infected bovine to test if ticks acquire and transmit *A. marginale in vitro*. After acquisition and transmission feedings, DNA was extracted and quantitative PCR was performed. For acquisition of *A. marginale*, the ticks were allowed to feed for 7 days. After acquisition feeding, *in vitro* fed ticks were dissected to harvest midguts and salivary glands. Acquisition fed ticks had a midgut infection rate of 92% with an average level of 2.7×10^6 *A. marginale* per midgut. The infection rate of salivary glands was 72% with an average level of 1.2×10^5 *A. marginale* per pair of salivary glands. For transmission, ticks were fed for a second time for 6 days on uninfected blood. Transmission fed ticks had a midgut infection rate of 88% with the average level of *A. marginale* similar to acquisition fed ticks. The infection rate of salivary glands was 72% with an average level of 3.7×10^6 *A. marginale* per pair of salivary glands. During the second feeding in the *in vitro* tick feeding system, blood samples were collected daily. At day 4, PCR detected that *A. marginale* had been secreted into the *in vitro* tick feeding system. In conclusion, we demonstrated that *in vitro* fed ticks acquired *A. marginale* and bacteria replicated in tick salivary glands to the level sufficient for transmission.

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Comparison of chlortetracycline and oxytetracycline treatment regimens to clear bovine anaplasmosis

Kathryn Reif, Tippawan Anantatat, Michael Kleinhenz, Emily Reppert, Johann Coetzee

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Abstract

Bovine anaplasmosis is 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. Tetracycline antimicrobials are the only approved antimicrobial class for treatment and control of bovine anaplasmosis in the U.S. Specifically, chlortetracycline-medicated feeds are approved for the control of active anaplasmosis infection, while oxytetracycline injectable drug products are approved for the treatment of anaplasmosis. Both antimicrobials have been demonstrated effective in controlling acute anaplasmosis but whether either option effectively clears *A. marginale* infection is unclear. Producers living in endemic areas commonly administer CTC-medicated feed for four to six months per year to control active anaplasmosis, while other producers may repeatedly administer oxytetracycline in an effort to clear infection. To determine the effect of chlortetracycline versus oxytetracycline treatment on anaplasmosis infection status and clearance, groups of cattle treated with FDA-approved hand-fed chlortetracycline dosages and FDA-approved oxytetracycline dosages were compared. This study describes the results of both experimentally infected and treated cattle, as well as, treatment of infected cattle from a naturally-endemic privately-owned herd in Virginia. As tetracycline antimicrobials are the only FDA-approved antimicrobials to treat *A. marginale* infection, studies critically assessing the efficacy of this medically-important antimicrobial to control bovine anaplasmosis are needed to inform science-based policy recommendations and improve antimicrobial stewardship.

Vaccine-mediated protection against pulmonary Q fever in three animal models.

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Abstract

A formalin-inactivated, whole-cell vaccine (WCV/QVax) against Q fever is available in Australia but requires significant monitoring to exclude individuals with prior immunologic memory. We have used several approaches to generate novel vaccines with protection comparable to WCV/QVax, without any adverse reactions. A proteome-wide survey identified a collection of *Coxiella* immunodominant antigens detected in convalescent serum that were recombinantly expressed and coupled onto a gold nanoparticle adjuvant. An alternate approach isolated *C. burnetii* native antigens from an avirulent phase II strain as vaccine material (SolWCVII). Vaccine candidates from both approaches were evaluated for protective efficacy in mouse and guinea pig models of pulmonary Q fever. Analysis of immunologic response from animals following vaccination or infection identified antibody, particularly those of IgG2c subclass, and CD4⁺ T-cell responses as important correlates and components for protective immunity; evidenced by passive and adoptive transfer. Recombinant antigens coupled to gold nanoparticles demonstrated measurable protection compared with sham, but further protection was achieved using SolWCVII vaccine, comparable with WCV. Additionally, SolWCVII did not show significant hypersensitivity following vaccination in previously-sensitized hairless guinea pig and non-human primate (NHP) models. SolWCVII was also evaluated for protection in a recently developed NHP model of aerosol-delivered Q fever. Unvaccinated NHPs developed a febrile response and pneumonia, whereas QVax and SolWCVII vaccinated NHPs did not exhibit overt clinical signs of disease. We believe this is the first report of vaccine-mediated protection in a NHP model of *C. burnetii* aerosol challenge that did not require material from phase I *C. burnetii*.

Session 7: Host-Pathogen-Vector Interactions

Chair: Anders Omsland & Minal Mulye

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CBU_1276 is Required for Intracellular Replication and Resistance to Oxidative Stress by *Coxiella burnetii*, the Causative Agent of Q fever

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Abstract

Coxiella burnetii, the causative agent of zoonotic disease Q fever, is a Gram-negative bacterium that replicates inside macrophages within a highly oxidative vacuole derived from the phagolysosome. Screening of a transposon mutant library for replication within human cells identified a number of genes involved in intracellular replication, including *cbu_1276*. CBU_1276 is a putative short chain dehydrogenase and contains a predicted NADP binding site that could facilitate NADP(H) regeneration by *C. burnetii*, a key process for surviving oxidative stress. Functional complementation of the *cbu_1276* mutant and replication assays confirmed the importance of CBU_1276 for intracellular replication. Bioinformatic analysis identified a functionally conserved glycine residue at position 12 which serves as a NADP binding site. This glycine residue was mutated to alanine using site-directed mutagenesis. Purified, recombinant 6xHis-1276 reduced NADP to NADP(H) whereas 6xHis-1276 G12A is catalytically inactive. Furthermore, CBU_1276 contributes to resistance to oxidative stress as hydrogen peroxide sensitivity was increased in the absence of CBU_1276. Together, these findings indicate that loss of CBU_1276 reduces NADPH production and thus renders *C. burnetii* more sensitive to oxidative stress, resulting in a significant defect in the ability of the bacterium to grow inside cells. In addition, untargeted gas chromatography/mass spectrometry analysis revealed significant differences in the abundance of a number of central carbon metabolites in the mutant compared to both wild type and the complemented mutant when grown *in vitro*, suggesting that, despite normal growth in culture medium, loss of CBU_1276 caused a fundamental shift in central carbon metabolism.

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***Coxiella burnetii* Subversion of the Macrophage Unfolded Protein Response**

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Abstract

Coxiella burnetii is the causative agent of human Q fever that presents as acute or chronic disease with symptoms ranging from fever and fatigue to fatal endocarditis. *C. burnetii* is a Gram-negative, intracellular bacterium that replicates within an acidic, lysosome-like parasitophorous vacuole (PV) in human alveolar macrophages. *C. burnetii* uses a Dot/Icm Type IV Secretion System (T4SS) to deliver bacterial proteins into the host cytoplasm. Multiple T4SS effectors localize to and/or disrupt the endoplasmic reticulum (ER) and secretory transport, but their role in infection is unknown. During microbial infection, unfolded nascent proteins may exceed the folding capacity of the ER, activating the unfolded protein response (UPR) and reverting the ER to its normal physiological state. Some intracellular pathogens manipulate the UPR and/or ER-Associated Degradation (ERAD) pathways to promote survival and replication in host cells. Here, we show that UPR and ERAD pathway inhibitors antagonize *C. burnetii* growth in macrophages, indicating these processes are needed for efficient infection. We investigated the three arms of the UPR, and found that the PERK arm is activated during *C. burnetii* infection of human macrophages, eliciting increased levels of phosphorylated eIF2 α and increased production and nuclear translocation of the transcription factor ATF4. ATF4 normally drives expression of pro-apoptotic CHOP; however, results suggest *C. burnetii* prevents production and downstream apoptotic effects of CHOP. Collectively, our data indicate *C. burnetii* modulation of the UPR is required for intramacrophage growth.

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Expanded specificity of an antibacterial tick effector Dae2 enables inhibition of host and environmental microbes.

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Abstract

Reported cases of Lyme disease, caused by the transmission of *Borrelia burgdorferi* (*Bb*) through the bite of *Ixodes* ticks, have nearly tripled in the US in the past decade. In addition to Lyme, *Ixodes scapularis* is able to transmit at least 5 other human pathogens, escalating the need for novel control mechanisms. Biocontrol solutions that disrupt vector-pathogen interactions are effective for mosquito- and fly-borne diseases. We use *Bb* as a model of vector-borne disease to understand tick-pathogen interactions. Previously, we identified an amidase effector, Dae2, in *I. scapularis* that was horizontally acquired from prokaryotes. Dae2 and its ancestral bacterial homolog, Tae2, target and degrade the bacterial peptidoglycan, a crucial part of the cell wall. However, the substrate specificity of Dae2 is far more diverse than Tae2, suggesting an evolutionary advantage for the ability to target an array of bacterial cell walls. Dae2 is produced throughout the tick and is secreted in the saliva. *dae2* knockdown led to an increased load of *Bb* in the tick. Although Dae2 is able to cleave purified *Bb* peptidoglycan, Dae2 does not effectively lyse *Bb*, leading to the hypothesis that Dae2 is acting on other microbes. We thus probed Dae2 activity on a number of microbes that the tick may encounter in the environment or at the host-bite site. Surprisingly, we found that Dae2 is a strong lytic effector for common skin microbes, such as *Staphylococcus epidermidis*, suggesting Dae2 may indirectly affect *Bb* transmission by mediating tick-microbe interactions at the host bite site.

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The relevance of *A. phagocytophilum* adhesin binding domains to *in vivo* infection

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Abstract

Anaplasma phagocytophilum causes granulocytic anaplasmosis, a debilitating infection that can be fatal in the elderly and immunocompromised. It also afflicts animals, including dogs, horses, and sheep. No granulocytic anaplasmosis vaccine exists. Because *A. phagocytophilum* is an obligate intracellular bacterium, targeting microbial-host cell interactions that mediate cellular invasion can disrupt infection. We previously identified three *A. phagocytophilum* adhesins that are key for cellular invasion – OmpA, AipA, and Asp14 – and determined that antibodies targeting the binding domains of all three drastically reduces infection of host cells. Here, we examined these adhesins' relevance to *A. phagocytophilum* infection *in vivo*. C57BL/6J mice were immunized against a cocktail of KLH-conjugated peptides corresponding to the OmpA, AipA, and Asp14 binding domains with an alum adjuvant. Controls were non-immunized mice or mice injected with alum alone or alum plus KLH. An ELISA revealed that AipA and Asp14, but not OmpA antibodies were specifically elicited in all mice of the KLH-adhesin peptide-immunized group. Nonetheless, pooled sera from these mice inhibited *A. phagocytophilum* infection of HL-60 cells. All mouse groups were challenged followed by measure of the peripheral blood bacterial load on days 4, 8, 12, 16, 21, and 28. Compared to controls, the *A. phagocytophilum* burden was reduced several-fold in mice in which an anti-AipA/Asp14 immune response had been elicited. Thus, AipA and Asp14 are critical for *A. phagocytophilum* to productively infect mice and immunization against their binding domains elicits a protective immune response.

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Mice lacking interferon signaling as robust models to investigate *Rickettsia parkeri* pathogenesis and host response

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Abstract

Current mouse models are of limited utility for investigating pathogenesis of the obligate cytosolic human pathogen *Rickettsia parkeri* because mice naturally restrict bacterial growth and develop limited or no overt infection-related symptoms. Towards developing a useful murine model, we have observed that C57BL/6 and 129 mice carrying mutations in the genes encoding receptors for both type I and type II interferon (*Ifnar^{-/-}Ifngr^{-/-}*) succumb to *R. parkeri* upon intravenous or intradermal inoculation. Furthermore, intradermal infection results in a necrotic lesion at the site of infection, mimicking human disease. We propose that this mouse strain can serve as a model for studying *R. parkeri* pathogenesis at the cell, tissue, and organismal levels in vivo. We are now developing this mouse model as a tool for investigating the role for *R. parkeri* virulence genes, including *sca2*, which is required for actin-based motility and cell-to-cell spread. We have observed that *sca2* mutant bacteria cause reduced vascular damage and mortality, and are reduced in their ability to disseminate from the dermis to deeper tissues. This illustrates the importance of actin-based motility for *R. parkeri* dissemination within a mammalian host. Collectively, our findings establish the *Ifnar^{-/-}Ifngr^{-/-}* mouse as a robust model to investigate *R. parkeri* pathogenesis and host response.

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Modifications of outer membrane protein B protect *Rickettsia parkeri* from ubiquitylation

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Abstract

The interplay between *Rickettsia* and host intracellular immune pathways, such as antimicrobial autophagy, is not well understood. We previously discovered that outer membrane protein B (OmpB) is required to protect *Rickettsia parkeri* from ubiquitylation and subsequent degradation via autophagy in immune cells. We also observed that OmpB-deficient bacteria lack a capsular-like layer. Nevertheless, the molecular mechanisms by which OmpB protects *R. parkeri* from ubiquitylation remain unclear. Towards elucidating these mechanisms and identifying other pathways that inhibit ubiquitylation of bacteria, we screened 250 *R. parkeri* transposon mutants for increased ubiquitylation via immunofluorescence microscopy. The screen identified mutant strains with transposon insertions in four different genes. Two of the mutations disrupted the *pkmt1* and *pkmt2* genes encoding for protein-lysine methyltransferases (PKMTs) that are known to methylate lysine residues in OmpB. Two other mutations disrupted the *wecA* and *rmID* genes that presumably are required for O-antigen biosynthesis and may also be important for biogenesis of the capsular-like layer. To determine whether OmpA or OmpB were ubiquitylated in the mutant strains, we performed pull-downs from cells expressing His-tagged ubiquitin, accompanied by OmpB and OmpA western blotting. We found that the PKMTs were each required to protect both OmpB and OmpA from ubiquitylation, whereas the O-antigen primarily protected OmpA. Together, these results suggest a critical role for post-translational modification of OmpB, and O-antigen biosynthesis, in protecting an intracellular bacterium from ubiquitylation. Future work to elucidate how modification of surface-exposed molecules protects *R. parkeri* may reveal general principles for pathogen avoidance of host intracellular immune pathways.

An *Orientia tsutsugamushi* effector modulates cellular levels of the MHC-I transactivator NLRC5

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Abstract

The major histocompatibility complex class I (MHC-I) pathway is crucial for immune defense against intracellular pathogens. We recently reported that the obligate intracellular bacterium *Orientia tsutsugamushi* inhibited MHC-I biosynthesis by reducing cellular levels of the MHC-I gene transactivator, NLRC5. Here, we link the molecular basis of this novel strategy to Ank5, a T1SS effector that carries an N-terminal ankyrin repeat domain and a C-terminal F-box, the latter of which is a eukaryotic-like domain known to co-opt the SCF1 (Skp1-Cullin1-F-box) E3 polyubiquitin ligase complex. Yeast two-hybrid identified NLRC5 as a putative Ank5 binding partner and co-immunoprecipitation (CoIP) verified the interaction. The ability of Ank5 to bind NLRC5 was ankyrin repeat-, but not F-box-dependent. Guided by molecular docking predictions, CoIP using alanine-substituted Ank5 proteins identified two residues of the ankyrin repeat region that are key for binding NLRC5. Ectopically expressed Ank5, but none of multiple other *O. tsutsugamushi* Anks reduced NLRC5 and MHC-I levels to phenocopy infection-associated events. The ability of Ank5 to do so is reliant on having an intact F-box and is ablated in cells in which Skp1 has been knocked down using siRNA. These data imply that Ank5 binds NLRC5 via specific residues in its ankyrin repeat domain and promotes reduction of the transactivator in an F-box-dependent manner, presumably by guiding K48-polyubiquitination that destines it for 26S proteasomal degradation. These findings expand knowledge of how *O. tsutsugamushi* and potentially other intracellular pathogens dysregulate the MHC-I-dependent immune response.

**30th Meeting of the
American Society for Rickettsiology:
Rickettsial Diseases at the
Vector-Pathogen Interface**



**June 8-11, 2019
El Dorado Hotel,
Santa Fe, New Mexico**



Tuesday, June 11, 2019
Abstracts

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.

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Identification and characterization of T4SS substrates in *Wolbachia pipientis*

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Abstract

Wolbachia are alpha-proteobacteria, part of the anciently intracellular *Anaplasmataceae*, and related to the important human pathogens *Anaplasma*, *Rickettsia* and *Ehrlichia*. However, *Wolbachia* do not infect mammals, but instead are well known for their reproductive manipulations of insect populations. In order for *Wolbachia* to establish itself in an insect population it must invade host cells, persist during infection, and be transmitted to the next generation. Like all intracellular bacteria, *Wolbachia* need to manipulate the host cell to invade and persist. Many microbes accomplish this via secretion systems. All *Wolbachia* symbionts encode a functional type IV secretion system (T4SS), which is expressed by *Wolbachia* within its native host. What has eluded researchers until recently is the identification of proteins secreted by *Wolbachia*. These proteins, referred to as effectors, often act to manipulate or usurp host cell processes in order to promote bacterial infection. We used a polyphasic approach to identify and characterize *Wolbachia* effectors. We identified candidate *Wolbachia* effectors using bioinformatics, then, we expressed the effectors in the model eukaryote (*Saccharomyces*), identifying proteins that induce a growth defect upon overexpression. We discovered that predicted effectors are coregulated with the T4SS using RNA-seq in the native host. Finally, we show that one of these predicted effectors is secreted via a heterologous assay, is found in the host cytosol, binds to host proteins, and facilitates maternal transmission. Our results pave the way for future work on mechanisms of symbiosis in this enigmatic symbiont.

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A conserved, potential nucleomodulin in *Rickettsia* species

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Abstract

Pathogenic bacteria employ specialized molecular secretion systems to deliver virulence factors and/or effectors to subvert host defense strategies. Despite a pattern of evolution via genome reduction, *Rickettsia* species encode and maintain effector delivery mechanisms, including components of a VirB/D-based type IV secretion system, yet only a few rickettsial effectors have so far been recognized and functionally characterized. By RNA sequencing and quantitative RT-PCR, we have identified abundant expression of RC0103, currently annotated as a hypothetical protein, during *in vitro* infection of human microvascular endothelial cells (HMECs) with *R. conorii* and significant upregulation of RC0103 expression at 24h when compared to 3h post-infection (38±5 copies *versus* 11±4 copies/bacterium). Sequence analysis revealed the presence of a bona fide bipartite nuclear translocation signal and existence of RC0103 homologs in all *Rickettsia* species. Circular dichroism spectroscopy of recombinant RC0103 suggested a unique antiparallel β -sheet secondary structure with approximately 28% left-twisted and 25% right-twisted sheets. Ectopic expression of RC0103 in HMECs revealed its translocation into the nucleus, yielding first evidence for its potential functional roles as a nucleomodulin. We next investigated the downstream effects of RC0103 nuclear translocation on the expression of 84 genes using an endothelial-specific PCR array and follow-up validation by quantitative RT-PCR. Our initial findings suggest increased expression of matrix metalloproteinases MMP1 and MMP2 and down-regulation of the tissue inhibitor of MMPs TIMP-1 in HMECs stably transfected with RC0103, suggesting a potential virulence factor-like activity involved in vascular dysfunction. Further in-depth studies into RC0103 secretion mechanisms and host transcriptome regulation are ongoing.

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A *Rickettsia typhi* phosphatidylinositol 3-kinase, RT0135, is an *rvh* type IV secretion system effector that modulates plasma membrane phosphoinositide metabolism during invasion and subverts cellular autophagy to establish its intracellular niche

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Abstract

Many invasive bacteria repurpose host phosphoinositide (PI) metabolism to induce phagocytosis, subvert intracellular trafficking, delay endosomal-lysosomal fusion, or avoid autolysosomal killing. These manipulative processes are often mediated by secretion system effectors. For *Rickettsia typhi*, the etiological agent of murine typhus, we previously characterized two *rvh* type IV secretion system (T4SS) effectors: RARP-2 targets the host endoplasmic reticulum, while RalF activates host Arf6 at the plasma membrane to facilitate PI 4-phosphate-5 kinase-mediated generation of PI 4,5-bisphosphate (PIP₂). PIP₂ accumulation at early phagocytic cup formation leads to downstream PI shifts throughout phagocytosis: class I PI3-kinase (PI3K)-mediated phosphorylation of PIP₂ to PI (3,4,5)-trisphosphate (PIP₃) for phagocytic cup closure, and ultimately class III PI3K-mediated generation of PI 3-phosphate (PI3P), the dominant PI on early endosomes. Accordingly, we hypothesized that additional rickettsial effectors might target host PI metabolism for its survival and pathogenesis. Indeed, immunoprecipitation of *R. typhi* lysate using an anti-RvHD4 (the *rvh*T4SS signal recognition protein) antibody revealed a novel PI3K domain-containing effector (RT0135). *In vitro* assays with recombinant RT0135 revealed both class I (PIP₂ to PIP₃) and class III (PI to PI3P) PI3K activities, a unique feature among characterized bacterial PI3Ks. *R. typhi* secretes RT0135 during host cell infection, with neutralization of RT0135 PI3K activity diminishing PIP₂-PIP₃ conversion and significantly reducing invasion. Furthermore, RT0135 binds Beclin-1 on autophagosomes later in infection, a likely safeguard against autophagolysosomal destruction. Collectively, our data suggest that *R. typhi* secretes a PI3K effector, RT0135, that facilitates intracellular growth by repurposing plasma membrane PI metabolism during invasion and subverting cellular autophagy later in infection

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Inhibition of *Ehrlichia chaffeensis* Infection by Cell-permeable Bicyclic Peptides that Bind Ehrlichial Type IV Secretion Effector Etf-1

Mingqun Lin, Amritendu Koley, Wenqing Zhang, Dehua Pei, Yasuko Rikihisa

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Abstract

Ehrlichia chaffeensis is an obligatory intracellular bacterium that causes human monocytic ehrlichiosis (HME), an emerging life-threatening infectious disease worldwide. *E. chaffeensis* encodes a type IV secretion effector protein, Ehrlichial translocated factor-1 (Etf-1), which is abundantly produced and secreted into infected cells. Etf-1 is critical for *Ehrlichia* infection of human monocytes by subverting and manipulating two important innate immune defense mechanisms against intracellular infection, cellular apoptosis and autophagy. Therefore, Etf-1 can serve as a potential therapeutic target for HME, since currently, the only drug is the broad-spectrum antibiotic doxycycline and no vaccine exists. In this study, we screened a combinatorial library of cell-permeable bicyclic peptides (bicyclic CPPs), which feature an ensemble of random peptide sequences in the first ring and a family of cell-penetrating peptides in the second ring, for binding to Etf-1. Two rounds of screening of over 320,000 bicyclic CPPs identified 30 hits that bound to Etf-1, and among them, two peptides (B7 and 174-5) interacted with Etf-1 with relatively high affinity (K_d of 5.3 and 1.5 mM, respectively) by fluorescence anisotropy assays. Treatment of *E. chaffeensis* infected THP-1 cells showed that peptides B7 and 174-5 significantly blocked *Ehrlichia* infection in a dose-dependent manner, but a control peptide C17, which did not bind to Etf-1, had no effect on bacterial infection. Our results demonstrate the feasibility of developing cell-permeable macrocyclic peptides as Etf-1 inhibitors for potential treatment of diseases caused by obligatory intracellular bacteria.

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Modulation of NF- κ B signalling by a *Coxiella burnetii* eukaryotic-like effector protein

Melanie Burette, Julie Allombert, Ghizlane Maarifi, Sebastien Nisole, Karine Lambou, Matteo Bonazzi
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Abstract

Coxiella burnetii, the etiological agent of the worldwide emerging zoonosis Q fever, replicates inside host cells in large autolysosome-like compartments and persists by protecting infected cells from apoptosis and silencing the innate immune response to infection. Recent bioinformatics analysis led to the identification of 7 *Coxiella* effector proteins containing eukaryotic-like domains, potentially involved in protein-protein interactions, post-translational modification and chromatin rearrangements. Among these, NopA (for Nucleolar protein A), displays 4 regulation of chromatin condensation (RCC) domains, which are found in the eukaryotic Ran GEF RCC1. Similar to RCC1, NopA localizes at the nucleus of infected or transfected cells, it is found associated with the chromatin nuclear fraction, and uses the RCC domains to interact with Ran, a GTPase involved in the nucleocytoplasmic transport. Differently from RCC1 however, NopA accumulates at nucleoli and sequesters Ran, thus perturbing nucleocytoplasmic transport. Indeed, NopA disrupts the Ran GDP-GTP gradient by increasing Ran-GTP levels in infected or transfected cells leading to a defect in the nuclear import of the NF- κ B subunit p65. Accordingly, qRT-PCR analysis on a panel of cytokines has shown that cells exposed to the *Coxiella nopA::Tn* or *dotA::Tn* mutant strains present a functional innate immune response, as opposed to cells exposed to wt *Coxiella* or the corresponding *nopA* complemented strain. Thus NopA is a master regulator of the innate immune response allowing *Coxiella* to behave as a stealth pathogen.

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A small RNA that regulates pyrimidine and methionine metabolism and possibly type IV secretion system effector CvpD is necessary for establishing *Coxiella burnetii*'s intracellular niche during infection

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Abstract

Coxiella burnetii is an obligate intracellular gammaproteobacterium and zoonotic agent of Q fever. We previously identified 15 small non-coding RNAs (sRNAs) of *C. burnetii* that showed differential expression in large and small cell variants grown axenically and in infected host cells. One sRNA, termed *Coxiella burnetii* small RNA 12 (CbsR12), was highly expressed in both cell types *in vitro* and was the dominant non-tRNA/rRNA/tmRNA transcript during infection of cultured mammalian cells. Through a combination of *in vitro* and *in vivo* assays, we identified several targets of CbsR12. First, we show that CbsR12 binds to and *upregulates* translation of *carA* transcripts encoding carbamoyl phosphate synthetase A; an enzyme that catalyzes the first step of pyrimidine biosynthesis. Second, CbsR12 binds and *downregulates* translation of *metK* transcripts encoding S-adenosyl methionine (SAM) synthase, an essential component of the methionine cycle. Third, CbsR12 binds to *cvpD* transcripts, coding for a type IVB secretion system substrate, both *in vitro* and *in vivo* and is predicted to upregulate expression of the CvpD effector. Finally, we have established that CbsR12 is necessary for full expansion of *Coxiella*-containing vacuoles (CCVs) and is correlated with growth rate in a dose-dependent manner in the early phase of a host cell infection. This is the first characterization of a *trans*-acting sRNA of *C. burnetii* and description of a bacterial sRNA that regulates *carA* and *metK* expression. These results illustrate the importance of sRNA-mediated regulation in establishment of the intracellular CCV niche.

Initial structural characterisation of the DNA binding domain of AnkA from *Anaplasma phagocytophilum*

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Abstract

The obligate intracellular bacterium *Anaplasma phagocytophilum* reprograms mammalian host transcription at a genome-wide level. The type IV secretion effector protein AnkA is crucial in this reprogramming event. Structure prediction analysis has previously highlighted that AnkA comprises several modular ankyrin repeats, a domain known to mediate protein:protein interactions. In the host cell, AnkA has two fields of influence: in the cytoplasm where it interferes with signalling pathways, and in the nucleus where it alters host chromatin and down-regulates host defence genes. AnkA mediates these changes in gene expression via interaction with host proteins but has also been demonstrated to have DNA binding activity, both *in vitro* and *in vivo*, despite lacking any recognisable DNA binding domain. Similarly, DNA binding activity has been attributed to an ankyrin of *Ehrlichia chaffeensis*, which is also implicated in reprogramming of host cells. Thus, a role for the ankyrin repeat in DNA binding, an activity not normally attributed to this domain, seems to be conserved in the ankyrins of varying *Rickettsiales*. We sought to structurally characterise the AnkA protein and determine the protein features responsible for DNA recognition. The crystal structure of the N-terminus of AnkA, solved to 2.3 angstrom, is presented. Whilst this domain of AnkA contains multiple canonical ankyrin repeats, multiple variant repeats are present and give rise to unique structural features. Analysis of the surface of AnkA provides clues to interactions with both host DNA and protein ligands. These results will allow the design of hypotheses to directly test AnkA effector mechanisms.

Session 9: Omics

Chair: Joao Pedra & Shaun Wachter

Dual RNA sequencing reveals insights into the biology of *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi (Ot) is the causative agent of the severe vector-borne disease scrub typhus. Studies on this and other *Rickettsiales* species are hampered by their being obligate intracellular organisms that are mostly genetically intractable. Here we have used a dual RNAseq approach to study the mechanistic basis of host-pathogen interactions in Ot. First, we addressed the question: how are genes organised and regulated in Ot? Ot has an unusual genome with almost half the genome composed of repetitive DNA elements and with minimal collinearity in gene order between strains. This raises questions about operon structure and regulation which we have studied by combining RNAseq data with comparative bioinformatics. The repetitive genome of Ot is largely driven by the presence of multiple copies of a mobile DNA element (RAGE). Using RNAseq analysis combined with proteomics we found that antisense transcription plays an important role in regulation of RAGE-associated gene expression. Second, we addressed the question: what bacterial and host genes contribute to virulence? We compared two strains using mouse infection models and used a correlative approach to identify bacterial and host genes whose expression levels were associated with virulence. This led us to identify certain bacterial surface proteins and TPR/Ank effector proteins and also the host IL33-FAS network that may be important in virulence. Taken together this work illustrates how dual RNAseq approaches can lead to insights into the biology and host-pathogen interactions of a genetically intractable organism such as Ot.

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Transcriptomic Profiling of Pulmonary Gene and lncRNA Expression in a Murine Model of *Rickettsia conorii* Infection

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Abstract

Mediterranean spotted fever due to *Rickettsia conorii* is an acute febrile illness of humans that can cause significant morbidity or mortality, yet little is known about changes in the expression of genes and long noncoding RNAs (lncRNAs) determining the host responses to infection. We have investigated the transcriptional landscape of host lungs as a prominently affected organ system in an established murine model of infection by RNA-sequencing. Among the reads mapping to non-coding RNAs, a total of 206 and 277 were significantly up- and down-regulated, respectively, in comparison to mock-infected controls. Systematic analysis of these transcripts enabled identification of two potentially active and up-regulated enhancer lncRNAs NONMMUT013718 and NONMMUT024103, demonstrating potential for the regulation of protein-coding genes Id2 (inhibitor of DNA binding 2) and Apol10b (apolipoprotein 10b), respectively. Expression of both elncRNAs and their targets was significantly higher in the lungs of infected mice. Ingenuity Pathway Analysis further revealed a number of differentially expressed genes and upstream regulators. Notably, genes encoding for ubiquitin D, antimicrobial peptides, calgranulins, cyto/chemokines, and guanylate binding proteins were highly up-regulated, whereas those participating in hemoglobin biosynthesis and heme homeostasis were significantly down-regulated. As response regulators, nucleotide-binding oligomerization domain-containing protein 2 and killer cell lectin-like receptors were differentially expressed and gene clustering revealed eukaryotic initiation factor-2, oxidative phosphorylation, and ubiquitination as predominantly activated biological pathways. Collectively, this first global transcriptomic profiling highlights *R. conorii*-induced regulation of novel genes, lncRNAs, and host pathways, thorough investigation of which will strengthen our understanding of the pathogenesis of rickettsial infections.

3 -

Mechanisms of establishing infection in *Wolbachia*

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Abstract

Intracellular pathogens have evolved many mechanisms to infect hosts, manipulate their biology, and evade immune responses. *Wolbachia* are alpha-proteobacteria, related to the intracellular human pathogens *Anaplasma*, *Rickettsia*, and *Ehrlichia*. Unlike their close relatives, *Wolbachia* inhabit the cells of arthropods and nematodes and are primarily vertically transmitted via the maternal germline. However, horizontal transfer does happen in nature. Additionally, some *Wolbachia* inhibit pathogen replication and are artificially transferred into target hosts, such as mosquitoes, for use in vector control. Establishing *Wolbachia* in a novel host is not trivial. We know relatively little about the requirements for *Wolbachia* to establish new infections, nor do we understand how *Wolbachia* respond to such a drastic change in environment. Here, we use transcriptomic and molecular approaches to explore how the *Wolbachia* strain wMel responds to moving between host contexts. To identify changes in *Wolbachia* gene expression that are in response to host signals, we generated transcriptomes from *Wolbachia* that were either in association with *Drosophila melanogaster* cells, or isolated from host cells and maintained in a host-free state. We find that once removed from host cells, *Wolbachia* no longer produce essential components of their Type IV secretion system (T4SS). *Wolbachia* appear to modulate expression of their T4SS depending on host contact. Additionally, transcriptomics revealed a suite of other *Wolbachia* loci that are differentially expressed when *Wolbachia* are extracellular versus in association with the host. Results will generate a deeper understanding of how *Wolbachia* initiates symbioses and inform the use of *Wolbachia* in insect control.

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Histone H3 deacetylation in *ex vivo* human neutrophils infected by *Anaplasma phagocytophilum*

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Abstract

Background: In HL-60 promyelocytic leukemia cells, *Anaplasma phagocytophilum* (*Aph*) infection reprograms host gene expression via epigenetic manipulation. A significant gap of *Aph* research is a lack of genome-wide epigenetic reprogramming studies in neutrophils. Therefore, a manipulable human neutrophil-*Aph* model is highly desirable. Methods: *Aph* was isolated from supernatants of heavily infected HL-60 cells using minimum manipulation. Human peripheral blood neutrophils obtained from 20 mL of EDTA-anticoagulated healthy donor blood were infected with cell free *Aph* for 18 to 24h and assayed for infection rate. Chromatin from uninfected and infected human neutrophils was prepared and sheared to 150 to 300 bp for downstream Illumina sequencing. To determine the efficacy of chromatin immunoprecipitation (ChIP), we used anti-acetyl-histone H3 (acH3) and targeted promoter regions of host defense genes. A pilot qPCR study of immunoprecipitated DNAs determined fold change of promoter-bound acH3 to ascertain quality of the model and methodology. Results: *Ex vivo* infection of human neutrophils by *Aph* yielded infection rates as high as 22%, compared to <1% by prior methods and a median of 1% *in vivo*. ChIP-qPCR targeting selected host defense promoter regions showed decreased acH3 (median -2.4 fold; range -1.3 to -3,000) around 7 out of 8 promoters. Conclusions: *Aph* leads to deacetylation of histone H3 in human neutrophils at transcriptionally silenced host defense gene promoters. This *Aph ex vivo* human neutrophil model provides a key tool to study global chromatin architecture by ChIP-Seq/Hi-C/3C-Seq methods and to define the roles of cellular reprogramming in host-pathogen interactions and pathogen fitness.

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Now This Party's Jumping! Sequence, assembly and annotation of the cat flea *Ctenocephalides felis* (Bouché) genome.

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Abstract

Fleas are small flightless insect parasites of birds and mammals. As blood-feeders, fleas are the vectors of many serious human diseases, most notably bubonic plague, murine typhus, and cat-scratch disease. The unavailability of flea genome sequences has hindered research, especially molecular comparisons to other arthropod disease vectors. Coupling Illumina and PacBio sequencing with Hi-C scaffolding (Chicago and Dovetail) techniques, we generated a chromosome-level genome assembly for the cat flea, *Ctenocephalides felis*. Our 773.8 Mb assembly includes 654.0 Mb (85%) in nine scaffolds larger than 10 Mb, suggesting the *C. felis* genome likely contains nine chromosomes. Analysis of conserved arthropod single-copy genes supports a comprehensive and highly intact assembly covering the flea genome. Our analyses indicate a recent episodic burst of gene duplication representing 45% of CDS, as well as an inordinate number of tRNA genes (roughly four-fold higher than other insects). Additional taxonomic binning of 1.6 Gb of Illumina reads revealed 252.1 Mb of microbial sequence (dominated by *Proteobacteria*) from which two novel and divergent *Wolbachia* endosymbionts were fully assembled. Interestingly, two flea scaffolds encode *Wolbachia*-like cytoplasmic incompatibility (CI) antitoxin genes that are expressed (RNA-Seq and qPCR), lending insight into our recent hypothesis that *Wolbachia*-derived arthropod CI genes may impart defense against reproductive parasitism. The *C. felis* genome and its microbiome are valuable new resources for arthropod phylogenomics and vector biology.

5 -

MALDI-TOF MS for rapid identification of arthropod vectors of rickettsial agents in Europe and Africa

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Abstract

In the last few years, matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used for the protein-profiling identification of bacteria and other microorganisms. This has led to a revolution in clinical microbiology. More recently, MALDI-TOF MS has been recently applied by our group for the identification of several arthropod vectors, such as ticks, fleas, mosquitoes from laboratory, collection or field specimens. It has also been used to differentiate arthropods infected or not by rickettsiae. This communication aims to present our most recent developments of this technology in the field of rickettsial diseases in Europe and Africa.

Poster Session 2

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Molecular Detection of *Rickettsia* in American Dog Ticks Collected Along the Platte River in South Central Nebraska

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Abstract

Dermacentor variabilis is the predominant tick species in Nebraska and is presumed to be the primary vector of *Rickettsia rickettsii* associated with Nebraskans that have contracted Rocky Mountain spotted fever. Interestingly, cases of Rocky Mountain spotted fever in Nebraska have increased on a year over year basis, yet the prevalence of *D. variabilis* vectoring *R. rickettsii* has not been established for Nebraska. Here we sought to set a baseline for the prevalence of *D. variabilis* vectoring *R. rickettsii* and other spotted fever group (SFG) rickettsiae. Over a 3 year period, *D. variabilis* were collected along the Platte River in south central Nebraska. Individual tick DNA was analyzed using endpoint PCR to identify ticks carrying SFG rickettsiae. A total of 927 *D. variabilis* were analyzed by PCR and 38 (4.1%) ticks tested positive for SFG rickettsiae. Presumptive positives were sequenced to identify the *Rickettsia* species, of which 29 (76%) were *R. montanensis*, 5 (13%) were *R. amblyommatis*, 4 (11%) were *R. bellii*, and *R. rickettsii* was not detected. These data indicate that *R. rickettsii* is likely at a low prevalence in south central Nebraska and spillover of *R. amblyommatis* into *D. variabilis* is occurring likely due to the invasive lone star tick (*Amblyoma americanum*). In addition, our data suggest that *R. montanensis* and *R. amblyommatis* could be associated with the increase in SFG rickettsiosis in Nebraska. This information will be of value to clinicians and the general public for evaluating diagnosis of disease and risk associated environmental exposure, respectively.

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Dynamic Gene Expression of Cat Flea-Derived Salivary Gland-Secreted Factors during *Rickettsia felis* Infection

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Abstract

Hematophagous insect saliva contains multiple biomolecules with anticlotting, vasodilatory, and immunomodulatory activities. Moreover, while feeding on host organism, bloodsucking arthropods also secrete proteins to facilitate microbial survival, growth, and transmission. The cat flea, *Ctenocephalides felis*, is a recognized arthropod vector transmitting several human pathogens, including *Rickettsia* spp. The sialotranscriptome of the cat flea was already characterized; however, the exact role of salivary factors and the molecular interaction between flea-borne rickettsiae and their insect host are still largely unknown. We hypothesize that *Rickettsia felis* modulates gene expression in cat fleas, thus regulating biomolecules essential for the successful horizontal bacterial transmission. Therefore, we infected cat fleas with *R. felis* to analyze differential transcription of selected salivary gland-derived factors. In our study, newly emerged adult fleas were fed bovine blood for 14 days. For infection assay, *R. felis* (strain LSU) was propagated in cell culture. Female and male salivary glands were microdissected separately from both control and infected groups at different time points upon inoculation. Subsequently, total RNA was extracted from salivary glands and the transcripts obtained by cDNA synthesis were subjected to real-time qPCR for gene expression analysis. The relative abundance of transcripts, coding putative salivary secreted factors in the infected cat fleas, was calculated using $2^{-\Delta\Delta C_t}$ method with elongation factor 1- α as a reference gene and compared to the uninfected flea group. Based on the expression profiles, we suggest there are flea-specific compounds responsive to rickettsial infection. Their role in *R. felis* transmission is under current study.

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Role of *Sca4* in the Dissemination and Transmission of *Rickettsia parkeri* in *Amblyomma maculatum*

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Abstract

Rickettsia parkeri, a member of spotted fever group *Rickettsia*, is believed to disseminate to host tissue by a cell-to-cell spread mechanism which involves actin base motility to provide a physical force to spread. More recently, it has been demonstrated that *R. parkeri* uses an alternate mechanism for cell-to-cell spread by secreting effector *sca4* to modulate protrusion and engulfment by manipulating vinculin-dependent intercellular tension. However, the interactions of *sca4* in the tick infection model are unknown. Using *R. parkeri* lacking functional *Sca4* (*R. parkeri sca4::tn*), we compared replication, growth kinetics and cell-to-cell spread *in vitro*, in tick and mammalian cells. In order to assess the role of *Sca4* in dissemination in the tick host and the transmission *in vivo*, *Rickettsia*-free *Amblyomma maculatum*, the natural vector of *R. parkeri*, was exposed to wild type or *R. parkeri sca4::tn* bacteria. Individual tick saliva and tissues including salivary glands, mid gut and ovaries, were analyzed by qPCR and IFA at 3 and 7 days post exposure after blood meal acquisition. A portion of the ticks were allowed to feed to repletion and subsamples of each stage of F₁ from each cohort were assessed for transovarial and transstadial transmission by qPCR. Blood from vertebrate hosts was collected at the same time points the ticks were collected. Blood, testis, and skin were assessed for infection using ELISA and histopathology, respectively. The preliminary data suggest the absence of *Sca4* gene reduces *Rickettsia* infection in the tick host.

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Comparative proteomics of different developmental stages of the Rickettsiales *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi (Ot) is an obligate intracellular bacterium and the causative agent of the human disease scrub typhus. The cellular infection cycle of Ot lasts 5-7 days in adherent fibroblast cells, and we have recently described how the bacterium transitions through 5 distinct stages during this infection: early entry, pre-replicative, replicative, maturation and extracellular (described in a separate poster by Suparat Giengkam et. al.). Here, we have isolated Ot populations in three of these stages (replicative, maturation and extracellular) and compared their protein profiles using tandem mass spectrometry. We have identified proteins whose expression levels are correlated with specific stages, and these include specific surface autotransporter proteins and ankyrin repeat proteins upregulated in the maturation and extracellular stages, as well as a number of tRNA- and ribosome-related genes also upregulated in the stages. These findings provide testable hypotheses about the mechanisms that this obligate intracellular bacterium uses in transitioning to a dedicated extracellular state that is primed for infection of new host cells.

Anaplasma marginale infection dynamics in an endemic region

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Abstract

Anaplasma marginale, is a tick-borne, bacterial pathogen of cattle, which causes significant losses to producers worldwide. Cattle in high prevalence endemic regions of Central America are persistently infected with multiple strains of *A. marginale*. Multi-strain infections are important because they can provide selective pressure for altered transmission and virulence phenotypes. Multi-strain infections occur via co-infection, in which multiple strains are acquired prior to the development of an adaptive immune response, or superinfection, in which a strain is acquired after the development of the adaptive immune response. The latter scenario requires greater genetic diversity. Under natural transmission conditions, it is unknown if strains are predominantly acquired through co-infection or super-infection. Due to the high genetic diversity among *A. marginale* strains, we hypothesize that *A. marginale* strains are primarily acquired by superinfection. We will address this question by tracking the acquisition of *A. marginale* strains over time under natural transmission conditions in a region of Ghana with high *A. marginale* prevalence and high transmission pressure. Twenty-five bovine blood samples were collected from persistently infected animals maintained on pasture at the University of Ghana. Genotyping to determine the number of strains per animal was done using multi-locus sequence typing based of five outer membrane protein-encoding genes. Of these 25 animals, 75% (17/25) harbored 2 to 5 strains, demonstrating that animals are infected with multiple strains in this endemic region of Ghana. Next, we will introduce naïve animals into the herd and track the acquisition of strains through time to differentiate between co-infection and superinfection.

First detection and characterization of *Coxiella burnetii* in ixodid ticks from Somalia

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Abstract

We investigated 237 tick samples collected from camels from seven different locations in Somalia. Using different genomic targets we screened the samples for *Coxiella burnetii* and *Coxiella*-like endosymbionts. 140 samples were reactive in the IS1111-PCR. Using *icd*- and *com1*-PCR 45 positive tick materials could be identified. Due to the extremely low amount of specific DNA in most of the samples (ct higher than 35), we selected 4 samples with a higher DNA amount for further genomic classification. After confirmation of *C. burnetii* with 16S rRNA-PCR, two other genome targets – acute disease A-antigen (*adaA*) -PCR (negative result) and a QpRS-plasmid PCR (positive) were tested. In addition, multilocus variant analysis (MLVA) genomic typing with 14-markers was applied and it showed that the detected *C. burnetii* is probably a new genotype. Moreover, the identified *C. burnetii* is closely related to a *C. burnetii* strain from Saudi Arabia. This is the first report of the occurrence of *C. burnetii* in ticks collected from camels in Somalia showing that this query agent might be endemic in this country. Further analysis should focus on screening ticks, the host animals (camels) and humans because camel milk could be a relevant risk factor for humans to catch the *C. burnetii* disease Q fever.

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Transcriptome Profiling of Inflammasome-Mediated Responses in Macrophages to *R. australis* Infection

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Abstract

Rickettsiae are tick-borne, obligately intracellular bacteria that initially target mammalian macrophages in the skin after inoculation by the feeding tick. The specific mechanisms involved in the interactions of macrophages with rickettsiae remain incompletely understood. The inflammasome is an intracellular multiprotein complex that detects pathogenic microorganisms and sterile stressors. Our previous studies have shown that ASC inflammasome mediates host control of rickettsial infection in macrophages and in an animal model of rickettsial diseases. Here, we employed Illumina Next Generation Sequencing to characterize ASC inflammasome-specific transcriptional profiling of *R. australis*-induced responses in macrophages. Using Ingenuity Pathway Analysis software, we analyzed 3899 significantly modulated genes at 1 hour post infection (h.p.i.) and 1545 genes at 24 h.p.i. of *R. australis*-infected and uninfected macrophages from wild type (WT) and ASC-knockout mice. Among these significantly regulated genes, 93% of modulated genes were down regulated by *R. australis* in ASC-knockout macrophages at 1 h.p.i., compared to 32% in WT macrophage controls. Knocking out of ASC led to downregulation of transcriptional programs associated with Cell-To-Cell Signaling and Interaction, Inflammatory Responses, and Cellular Movement, suggesting that ASC inflammasome promotes these bio-functions at the transcriptional level in *R. australis*-infected macrophages at the very early stage of infection. Our data provide insight into mechanisms by which macrophages control intracellular rickettsial infection.

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Profile of Serum Exosomal MicroRNAs in Mice Infected with *Orientia tsutsugamushi*

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Abstract

Exosomes are 40-100 nm extracellular vesicles that carry proteins, lipids and nucleic acids. They are circulated in body fluids and play important roles in intercellular communication and modulation of immune responses during viral and bacterial infections. Certain serum exosomal microRNAs (miRNAs) have been identified as diagnostic biomarkers and functionally, they interact with various immune cells that affect pathogenesis and host-pathogen interactions. Little is known whether exosomal miRNAs are regulated during scrub typhus, a potentially lethal infection caused by *Orientia tsutsugamushi*. To test this, we infected CD-1 mice through intraperitoneal inoculation. Serum was collected at various time points and exosomes were isolated at 4, 7 and 14 days post infection. A custom quantitative PCR array covering 92 murine miRNAs was used to detect any miRNA changes. A total of 18 miRNAs were found to be significantly up- or down-regulated at least at one time point in comparison to the controls. Pathway enrichment analysis suggests modulation on multiple metabolic pathways, including fatty acid biosynthesis, lysine degradation and protein processing in endoplasmic reticulum. Interestingly, three miRNAs in the let-7 family (let-7a-5p, let-7d-5p and let-7g-5p) were consistently down-regulated at all time points and quantities of several other members also decreased, although lacking statistical significance. Besides their involvement in human cancer, let-7 miRNAs have been shown to impact host immune responses through regulations on toll-like receptors, cytokine release and NF-κB pathways. Our data revealed comprehensive regulations of serum exosomal miRNA during scrub typhus infection, which could influence both host metabolism and immune responses.

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Elimination of the *Coxiella burnetii* QpH1 Plasmid

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Abstract

Axenic growth of *Coxiella burnetii* dramatically enabled development of genetic tools that are now being used to define pathogen virulence determinants. Interestingly, all *C. burnetii* strains sequenced to date carry a large (~32 – 54 kDa), autonomously replicating plasmid or have chromosomally integrated plasmid-like sequences (IPS), suggesting that plasmid genes are important for infection. Seven genes on the QpH1 plasmid, carried by the reference Nine Mile strain, encode type 4B secretion system (T4BSS) effector proteins suspected as mediating virulence. Only two of these genes are conserved between other *C. burnetii* plasmids or IPS. To investigate the role of the *C. burnetii* plasmid in virulence, we developed a new *E. coli*-*C. burnetii* shuttle vector (pBR322-CAT-*sacB*-*tyrB*-QpH1ori) that contains the QpH1 origin of replication cloned into an *E. coli* plasmid containing the *tyrB* gene. *C. burnetii* production of TyrB in the presence of the tyrosine precursor 4-hydroxyphenylpyruvic acid (4-HPA) rescues the bacterium's natural auxotrophy for tyrosine. Introduction of pBR322-CAT-*sacB*-*tyrB*-QpH1ori into *C. burnetii* Nine Mile (phase II), followed by growth in tyrosine-deficient acidified citrate cysteine medium (ACCM)-D supplemented with 4-HPA, resulted in expulsion of the native QpH1 plasmid. The mutant strain grew normally in axenic medium, but had a severe growth defect in host cells. This study defines the critical nature of *C. burnetii* plasmid sequences in host cell parasitism and identifies a novel method of nutritional selection of genetic transformants. Complementation studies are currently under way to define virulence roles of specific plasmid and IPS genes.

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Identifying molecular determinants of *B. burgdorferi* transmission in ticks

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Abstract

Ticks are blood-feeding arthropods that serve as vectors for numerous human and animal pathogens including *Borrelia burgdorferi*, the causative agent of Lyme disease. Only a restricted set of tick species are competent *B. burgdorferi* vectors. *Ixodes* ticks can acquire *B. burgdorferi*, which colonizes the tick midgut and can be transmitted via saliva. A key bottleneck in the transmission process is dissemination from the tick midgut to the salivary glands, and only a small number of *B. burgdorferi* successfully cross this critical threshold. An understanding of the molecular drivers behind this small population of transmitted *B. burgdorferi* in the tick may help identify novel ways to disrupt this process. We hypothesize that successful dissemination within the tick depends on the intrinsic genomic content of *B. burgdorferi* as well as expression of key genes involved in *B. burgdorferi* mobility and tick immune evasion. We will compare genomic and transcriptomic differences between *B. burgdorferi* in tick midguts and salivary glands to determine molecular programs that differ between these tissues. We will then test whether particular genes are necessary for dissemination by infecting ticks with *B. burgdorferi* knockouts or knockdowns and monitoring dissemination as they feed on naïve mice. These experiments will help identify new targets for vector-focused methods of controlling the spread of Lyme disease.

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Genetic characterization of a new strain of *Ehrlichia chaffeensis* from an *Amblyomma tenellum* tick from South Texas.

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Abstract

Ehrlichia chaffeensis is the causative agent of human monocytotropic ehrlichiosis (HME), a disease that ranges in severity from mild to fatal infection. *E. chaffeensis* is maintained in a zoonotic cycle involving white-tailed deer (*Odocoileus virginianus*) as the main vertebrate reservoir and lone star ticks (*Amblyomma americanum*) as its principal vector. Through genomic analysis of human ehrlichial isolates and DNA sequences from deer and ticks, eight strains of *E. chaffeensis* have been identified previously and characterized having variable pathogenicity in a SCID mouse model. Previous studies have shown that differences in the sequence and in the number of repeats of the genes encoding tandem-repeat protein (TRP) 32 (earlier VLPT, the variable length PCR target gene) and TRP120 are useful for detection and differentiation among *E. chaffeensis* strains. Here, we report the first evidence of *E. chaffeensis* DNA from an unfed adult female *Amblyomma tenellum* tick (formerly *Amblyomma imitator*), which was collected during field work in South Texas. Genetic characterization through the amplification and sequencing of the genes 16S rRNA, *dsb*, *groESL*, TRP32 and TRP120, suggest a novel *E. chaffeensis* strain. This strain exhibits greater diversity than other strains of *E. chaffeensis* and suggests a role for *A. tenellum* as a possible vector of *E. chaffeensis*.

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The *Anaplasma phagocytophilum* Adhesin Asp14 Exploits Host Cell Surface Protein Disulfide Isomerase Activity to Promote Infection

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Abstract

Diverse intracellular pathogens rely on eukaryotic cell surface oxidoreductases to invade host cells. Pharmacologic inhibition of oxidoreductases is cytotoxic, making it impractical for treatment. Identifying and mechanistically dissecting microbial proteins that co-opt oxidoreductases could reveal novel targets for disrupting a common infection strategy. *Anaplasma phagocytophilum* invades neutrophils by an incompletely defined mechanism to cause the potentially fatal disease, granulocytic anaplasmosis. The bacterium's adhesin, Asp14, contributes to invasion by virtue of its C-terminus engaging an unknown receptor. Yeast-two hybrid analysis identified protein disulfide isomerase (PDI) as an Asp14 binding partner. Co-immunoprecipitation confirmed the interaction and validated it to be Asp14 C-terminus-dependent. PDI knockdown and antibody-mediated inhibition of PDI reductase activity impaired *A. phagocytophilum* infection of, but not binding to host cells. Infection in the face of PDI inhibition was rescued when the bacterial, but not host cell surface was chemically reduced with tris(2-carboxyethyl)phosphine (TCEP). TCEP also restored bacterial infectivity in the presence of an Asp14 C-terminus blocking antibody that normally blocks infection. *A. phagocytophilum* failed to productively infect myeloid-specific PDI conditional knock-out mice, marking the first demonstration of *in vivo* microbial dependency on PDI for infection. Mutational analyses identified Asp14 C-terminal residues that contribute to PDI binding. These data demonstrate that Asp14 binds and brings PDI proximal to thiols of *A. phagocytophilum* surface proteins that it reduces, which enables cellular and *in vivo* infection. Targeting the Asp14 C-terminus could benefit approaches to prevent/treat granulocytic anaplasmosis. A thematically similar approach could block infection by other intracellular microbes that exploit cell surface oxidoreductases.

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Clinical characterization of *Orientia tsutsugamushi* Boryong strain in rhesus macaques (*Macaca mulatta*)

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Abstract

Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi*, a gram negative intracellular bacteria. It is an endemic disease in Asia-Pacific region and northern Australia; however, recent studies have reported emerging region of scrub typhus outside the traditional areas. In South Korea, scrub typhus was the third infectious disease most frequently reported in 2012, and *O. tsutsugamushi* Boryong, which can cause complications in multiple organs, was the major strain of *O. tsutsugamushi* reported. In this study, we aimed to characterize clinical manifestation of *O. tsutsugamushi* Boryong infection in rhesus macaques. Three rhesus macaques were intradermally inoculated with *O. tsutsugamushi* Boryong strain at dose 5×10^8 Focus Forming Unit (FFU)/ml. All *O. tsutsugamushi*-inoculated animals developed classical scrub typhus signs including eschar formation, regional lymphadenopathy, thrombocytopenia, elevation of body temperature, and bacteremia. By day 5 post inoculation, eschar was observed on both left and right thighs. Stage ranged from day 5-14 correlated with temperature curve. In addition, the reduction of appetite and activity was observed during the fever period. The serum level of alkaline phosphatase (ALP) and C-reactive protein (CRP) were increased on day 7 post-inoculation. These clinical signs are similar to those reported for human infection. Therefore, we conclude that rhesus macaque model of Boryong strain can be used for further experimental studies of scrub typhus and vaccine evaluation.

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***Rickettsia philipii* Infection in Mammalian Cells and Expression of Unique Genes Encoded by a Chromosome Insert**

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Abstract

Rickettsia philipii is the etiological agent of Pacific Coast tick fever, an emerging febrile illness manifesting with an eschar. *Rickettsia philipii* genome contains 11 unique genes encoded on a 19-kb insert absent in *Rickettsia rickettsii*. Our goal was to compare transcription of these 11 unique genes during *R. philipii* infection in two mammalian cells. VERO E6 cells and human microvascular endothelial cells (HMEC-1) were infected with *R. philipii* 364D and harvested at different time points. Culture supernatant was used to measure release of lactate dehydrogenase (LDH). Total RNA was extracted using the Zymo Quick-RNA protocol. Equal amounts of RNA were treated with DNase, and cDNA was generated using iScript reverse transcriptase. Detection of cDNA was performed using EvaGreen PCR with gene specific primers amplifying 100-137 bp fragments. Changes in each gene's expression were calculated using $2^{-\Delta\Delta C_t}$ method and normalized to the expression of *Rickettsia* 16S rRNA gene. *Rickettsia philipii* growth in VERO and HMEC-1 caused visual cytopathic effects starting at 72 hr post infection, and increasing leakage of cytoplasmic LDH between 24 hr and 96 hr of infection. These observations directly correlated to a continuous increase in transcription of *R. philipii* OmpA gene. Transcription of four genes encoded by the chromosome insert was upregulated between 3 and 48 hr in both cell lines. Transcription levels of HisK gene differed between VERO and HMEC-1. The role and contribution of the four upregulated genes to pathogenesis of *R. philipii* infection is being examined.

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Surveillance of Tick-borne Pathogens in Louisiana

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Abstract

Tick-borne diseases are a human and animal health concern for Louisiana due to the warm climate which provides year-long tick activity. Surveillance of companion animals is a useful resource to evaluate the distribution of tick-borne diseases and potential threats to human health due to their close association and cohabitation with humans. An ongoing surveillance partnership with the Louisiana Department of Health (LDH), Office of Public Health, Infectious Disease Epidemiology Section collects ticks from shelter animals and domestic pets to evaluate potential exposure to tick-borne pathogens in the human population. Ticks from each of the nine LDH administrative regions were screened for *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, SFG *Rickettsia* spp., *Bartonella henselae*, and *Borrelia burgdorferi* using qPCR. Subsamples of PCR-positive ticks were selected and portions of species-specific genes were sequenced to identify agents. From October 2018-March 2019, the species of ticks that have been recovered include (in descending order): *Ixodes scapularis*, *Amblyomma maculatum*, *Dermacentor variabilis*, and *Rhipicephalus sanguineus*. To date, 93% (188/202) of ticks are infected with SFG *Rickettsia* spp. Of those samples, *Rickettsia parkeri* and rickettsial endosymbionts have been identified. Other Rickettsiales or tick-borne pathogens have been identified, including *Anaplasma* and *Ehrlichia*. A surveillance project of this magnitude has not been conducted in the State of Louisiana for at least 20 years and the data gathered from this survey will aid public health and research professionals concerning tick-borne diseases.

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A genome-wide siRNA screen to study host factors involved in the early stages of infection by *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi is the causative agent of scrub typhus. This pathogen has been known for over a century, however, there are many aspects of its biology that are still unclear including molecular details of the host-pathogen interaction. *O. tsutsugamushi* is an obligate intracellular bacterium that enters into host cells using the endosomal pathway via a clathrin-mediated zipper-like mechanism. The bacteria subsequently escapes from the late endosome into the cytosol via an unknown mechanism. The bacteria in the cytosol triggers the autophagy defence system of the host cell but surprisingly it is able to evade autophagy and survive. The cytosolic bacterium then employs host microtubule filaments to traffic to the perinuclear membrane area where it undergoes growth and replication. Here we describe the optimization and early results from a siRNA-based genome wide screen to search for host factors important in early stages of infection into naive HeLa cells. We are using a high-throughput high-content imaging approach combined with machine learning algorithms in order to quantify morphological changes in both bacteria and host cells in the presence of siRNA inhibition of host cell targets. Our screen is based on a single infection time point 30 hours post infection, by which point bacteria are typically located in the perinuclear region. Hits in our screen will report on changes in the position or morphology of bacteria, and these will include perturbations in multiple stages including bacterial entry, endosome escape, autophagy escape and intracellular translocation.

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***Anaplasma phagocytophilum* Infection Involves Cholesterol-driven Association of NPC1 and Flotillin 2**

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Abstract

Anaplasma phagocytophilum is an obligatory intracellular bacterium that causes human granulocytic anaplasmosis, an emerging life-threatening infectious disease. *A. phagocytophilum* is a cholesterol-dependent bacterium that acquires cholesterol from host cells. Previous studies showed cholesterol-binding membrane proteins, Niemann-Pick type C1 (NPC1) and Flotillin (FLOT) are required for *A. phagocytophilum* infection. However, detailed mechanism is unknown. Here, we found that intraluminal localization of FLOT2 in NPC1-containing vesicles in both *A. phagocytophilum*-infected and uninfected mammalian cells, and physical interaction of FLOT2 with NPC1 proteins by pull-down assay. A loss-of-function mutant, NPC1(P691S), which contains an amino acid substitution in the sterol-sensing domain of NPC1 for intracellular cholesterol transport, not only reduced the colocalization and interaction of FLOT2 and NPC1, but also reduced *Anaplasma* infection. CRAC (cholesterol recognition/interaction amino acid cholesterol-binding) domain mutant of FLOT2 did not interact with NPC1. The cholesterol-sequestering agent methyl- β -cyclodextrin that inhibits *A. phagocytophilum* infection reduced NPC1 and FLOT2 interaction. Ezetimibe, a plasma cholesterol-reducing drug by decreasing cholesterol uptake by intestinal epithelial cells, effectively inhibited *A. phagocytophilum* infection in dose- and time-dependent manners by dissociating the interactions between NPC1 with FLOT2. Pre-treatment of host cell-free *A. phagocytophilum* with ezetimibe did not significantly abolish infection, confirming that ezetimibe does not directly inhibit *A. phagocytophilum*. These data indicate NPC1 and FLOT2 interaction is dependent on cellular cholesterol, and suggest this interaction is required for *A. phagocytophilum* infection through cholesterol acquisition

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Role of microRNAs in apoptosis inhibition during *Coxiella burnetii* infection

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Abstract

Inhibition of apoptosis, a programmed cell death, is a critical component of *Coxiella burnetii*'s pathogenicity. However, nothing is known about the roles host microRNAs (miRNAs), an important class of non-coding RNAs that regulate gene expression, potentially play in *C. burnetii* infection. In this study, we investigated the function of miRNAs during *Coxiella* infection, and show that they contribute to the subversion of host cell apoptosis. Through transcriptome profiling we explored miRNAs and messenger RNAs (mRNAs) in human monocytic leukemia cell line (THP-1) infected with wild-type (WT) *C. burnetii* or a strain (DotA) that has a non-functional Type 4 Secretion System. Comparative analyses of the differentially regulated miRNAs and mRNAs revealed 49 miRNAs potentially targeting 2766 mRNAs in WT-infected cells, whereas 98 miRNAs likely targeting 2629 mRNAs were identified in DotA-infected cells. Forty-three miRNAs that probably target 2409 protein-coding genes were common to both infections. Subsequent analysis of the paired miRNA-mRNA datasets showed that 16 miRNAs were likely critical for the inhibition of apoptosis observed during *Coxiella* infection e.g. via upregulation of antiapoptotic BCL2 family members. Furthermore, a few of these apoptosis-modulating miRNAs may also be involved in the regulation of nutritional immunity through processes such as metabolic privation of essential amino acids and iron restriction. Interestingly, a significant downregulation of DICER1 antisense RNA 1 was observed in infected cells, suggestive of increased cytoplasmic maturation of miRNAs during *Coxiella* infection. Collectively, our data indicate a role for miRNAs in the suppression of apoptosis by *C. burnetii*.

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The Effect of Extended Laboratory Propagation on The Genome and Virulence of *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi is the causative agent of scrub typhus disease, an acute febrile illness in humans with a high incidence in rural areas of Southeast Asia. As an obligate intercellular and slow growing bacterium, *O. tsutsugamushi* propagation involves extended passage in cultured cells. Some bacteria have been shown to decrease virulence following prolonged culture in the laboratory due to adaptation to those conditions. This has never been measured in *O. tsutsugamushi*. Published genome sequences of multiple *O. tsutsugamushi* strains revealed a high degree of recombination between different isolates. The mechanism and timescale of this recombination is unknown, and could occur during laboratory propagation. Therefore, this study aimed to determine the effect of long term growth on (i) the growth and virulence of *O. tsutsugamushi* and (ii) the genome of *O. tsutsugamushi*. In the present study, the two clinical strains UT76 and TM4942 isolated from patients in Thailand and Laos respectively, were subjected to extended laboratory propagation. We measured the effect of laboratory propagation on growth rate in culture and virulence in a mouse model. Long read genome sequencing was also carried out before and after extended propagation in order to track any change in the bacterial genomes of the isolates. The findings of this work will be presented here.

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Susceptibility of *Rickettsia rickettsii* to Tigecycline

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Abstract

Doxycycline is the drug of choice for all rickettsioses. In Colombia, where Rocky Mountain spotted fever is endemic, intravenous doxycycline is not available for use in those with life-threatening disease. Another tetracycline-like parenteral antibiotic, tigecycline, is available in Colombia, but its effectiveness against *R. rickettsii* is unknown. We aimed to determine the susceptibility of *R. rickettsii* to tigecycline *in vitro* and *in vivo*. To determine the minimum inhibitory concentration of tigecycline, *R. rickettsii*-inoculated Vero cells were incubated with medium containing tigecycline. At various time points, monolayers were collected and *R. rickettsii* was quantified via qPCR. The growth of *R. rickettsii* was inhibited in the presence of ≥ 0.5 $\mu\text{g/ml}$ of tigecycline. To determine the effectiveness of tigecycline *in vivo*, guinea pigs were inoculated with *R. rickettsii*. Five days after inoculation, they were treated twice daily with tigecycline 3.75 mg/kg or doxycycline 5 mg/kg. Treated animals improved, while untreated controls remained ill. Tissues were collected for qPCR on day 8. Median bacterial loads in the tigecycline group were less than in untreated animals: liver (0 versus 2.9×10^4 copies/mg), lung (0 versus 8.3×10^3 copies/mg), skin (2.5×10^2 versus 2.2×10^5 copies/mg), spleen (0 versus 1.3×10^4 copies/mg), and testes (0 versus 1.0×10^5 copies/mg). There were no significant differences in the bacterial loads between doxycycline-treated versus tigecycline-treated guinea pigs. These data indicate that tigecycline is effective against *R. rickettsii* in cell culture and in an animal model of RMSF.

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Developing an ELISA as a diagnostic assay for the detection of *Rickettsia felis*

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Abstract

Since its recognition in 1994 as the causative agent of human flea-borne spotted fever, *Rickettsia felis* has been reclassified from the spotted fever group (SFG) *Rickettsia* to the transitional group (TRG) based on genomic and biological differences. However, *R. felis*-infected hosts still share high serological cross-reactivity to the SFG and common clinical signs associated with other rickettsioses, causing common misdiagnoses. Like other insect-borne rickettsioses, *R. felis* infection of hosts has the potential to occur through infectious flea feces. Thus, this novel route of infection could provide alternative *R. felis*-specific antigenic profiles different from that of the SFG. ELISAs are relatively inexpensive, allow large quantities of samples to be analyzed at once, and are easily standardized. Thus, this study aimed to develop an ELISA and to determine its sensitivity in differentiating *R. felis* from the SFG *Rickettsia*. Sera collected from BALB/c mice injected with *R. felis*-infected cat flea feces, *R. felis* cultured in ISE6 cells, or *R. parkeri* cultured in Vero cells (used for its known cross-reactivity with *R. felis*) were exposed to *R. felis* cultured in ISE6 cells and *R. felis*-infected cat flea feces antigens to assess cross-reactivity. Results revealed that SFG antibodies had different reaction profiles between *R. felis*-infected cat flea feces and cultured *R. felis* antigens. The differential antibody recognition between these two antigens indicates that this novel route of *R. felis* transmission to a vertebrate host could provide the capability to differentiate flea-borne spotted fever from SFG infections serologically.

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Inhibition of *Ehrlichia chaffeensis* Infection by Intracellular Nanobodies that Bind *Ehrlichial* Type IV Secretion Effector Etf-1

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Abstract

Ehrlichia chaffeensis is an obligatory intracellular bacterium that causes human monocytic ehrlichiosis, an emerging life-threatening infectious disease worldwide. *E. chaffeensis* type IV secretion effector protein, Ehrlichial translocated factor-1 (Etf-1), is critical for *E. chaffeensis* infection of human monocytes by subverting and manipulating two important innate immune defense mechanisms against intracellular infection, mitochondria-mediated cellular apoptosis and autophagy. Therefore, Etf-1 can serve as a potential target for anti-Ehrlichial therapy. In this study, we developed nanobodies against Etf-1 to neutralize *E. chaffeensis* infection. After llama immunization with Etf-1 protein, peripheral blood lymphocytes were isolated and then used to prepare cDNA. Nanobody fragments were amplified from the cDNA and then ligated into the pMECS phage display vector, and transformed into *E. coli* TG1 strain to create a nanobody library of 2.32×10^{10} transformants. After recombinant phages were rescued and amplified with M13 helper phage superinfection, one round of phage display selection was performed using denatured and non-denatured recombinant Etf-1. Twenty-four nanobodies against Etf-1 were obtained, and recloned into mammalian expression vector. Transfection of mammalian host cells showed that nanobodies D1 and D7, which blocked mitochondria localization of Etf-1, also reduced *E. chaffeensis* infection; whereas the control nanobodies D3 and D29, which did not bind Etf-1 or affect its mitochondria localization, had no effects on bacterial infection. Our results demonstrate the development of novel nanobodies that interfere with Etf-1 functions, and will overcome current barriers to advance mechanistic research and therapeutic treatments of diseases caused by obligatory intracellular bacteria.

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New labelling approaches lead to new insights into the biology of Rickettsiales bacteria

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Abstract

Rickettsiales bacteria are obligate intracellular alpha-proteobacteria which live inside mammalian and arthropod hosts. These include *Orientia*, *Rickettsia*, *Ehrlichia* and *Anaplasma* species which cause various diseases in animals and humans. Studies on the cell biology of these organisms is complicated by the fact that they can only be grown inside living eukaryotic cells, and by the fact that most of species remain genetically intractable. In order to circumvent these challenges, we have begun using a range of small molecule chemical probes in order to study different aspects of the biology of their biology. Here we will present recent developments on the use of chemical probes to study protein synthesis, transcription, and peptidoglycan biosynthesis in Rickettsiales. First, we have used a clickable, non-toxic methionine analog probe which readily incorporates into newly synthesised proteins. This allows metabolically active bacteria to be visualised by fluorescent microscopy. Second, we have used single cell RNA fish to study the transcription of specific genes within populations of intracellular bacteria. This allows us to study differences in gene expression within a population and at different times after infection. Finally, we have used D-amino acid probes to label the peptidoglycan cell wall of some Rickettsiales species. This has allowed us to determine the presence and abundance of peptidoglycan in different species and at different times during the intracellular infection cycle. By using these methods to visualise Rickettsiales, we can begin to understand the complicated life cycle of these intracellular bacteria and further investigate important questions about their biology.

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Characterizing Early Stages of Human Alveolar Infection by the Q Fever Agent, *Coxiella burnetii*

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Abstract

Human Q fever is caused by the intracellular bacterial pathogen *Coxiella burnetii*. Q fever presents with acute flu-like and pulmonary symptoms, and can progress to chronic, severe endocarditis. After human inhalation, *C. burnetii* is engulfed by alveolar macrophages and transits through the phagolysosomal maturation pathway, resisting lysosomal insults to form a parasitophorous vacuole (PV) in which to replicate. Previous studies showed that *C. burnetii* replicates efficiently in primary human alveolar macrophages (hAMs) in *ex vivo* human lung tissue. Although *C. burnetii* replicates in most cell types *in vitro*, the pathogen does not grow in non-hAMs in human lung tissue. Here, we investigated the interaction between *C. burnetii* and other pulmonary cell types apart from the lung environment. *C. burnetii* formed a prototypical PV and replicated efficiently in human pulmonary fibroblasts and airway, but not alveolar, epithelial cells. Atypical PV expansion in alveolar epithelial cells was attributed in part to defective recruitment of autophagy-related proteins. Further assessment of the *C. burnetii* growth niche showed that macrophages mounted a robust IL-8, neutrophil-attracting response to *C. burnetii* and ultimately shifted to an M2-polarized phenotype characteristic of anti-inflammatory macrophages. The *ex vivo* lung tissue platform is currently being used to investigate the importance of the transcription factor Nrf2 in down-regulating oxidative stress during *C. burnetii* growth in macrophages. Collectively, these results provide enhanced understanding of the unique *C. burnetii*-lung dynamic and further establish the primary lung tissue platform for modeling early stages of *C. burnetii* infection.

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Persistence of *Coxiella burnetii* following an abortion storm on a small Texas goat farm

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Abstract

In the spring of 2018, a Q fever outbreak occurred on a small family farm of 100-200 goats in Texas, resulting in losses of over 50% of offspring. Q fever is caused by the bacterial pathogen, *Coxiella burnetii*, and many cases and outbreaks of Q fever in the US are associated with goats. A sampling project was started to evaluate the long-term outcome of an infected farm. In September 2018, 125 goats were sampled and tested by PCR to determine whether *C. burnetii* was actively being shed. Serum was collected from 100 of these goats to measure the presence of anti-*C. burnetii* antibodies. Additionally, 43 environmental samples were analyzed for the presence of *C. burnetii* DNA. All goats tested positive for the presence of anti-*C. burnetii* antibodies, with titers ranging from 16 to 131,072. The highest titers were observed in does and kids. *C. burnetii* DNA was detected in 28 of 109 vaginal swabs. Fecal swabs were negative from the 14 bucks and wethers sampled, but were positive from the two male kids tested. *C. burnetii* DNA was identified in 36 of the 43 environmental samples, with the most concentrated samples collected from the birthing pens. These findings demonstrate persistence of *C. burnetii* in animals and the environment months after an abortion storm, despite antibiotic treatment. Furthermore, our findings suggest that within a herd, does and kids are the major reservoir for *C. burnetii*. Future repeat sampling will provide information about persistence in the herd and environment.

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Searching Algorithm for Type IV Effector proteins (S4TE) 2.0: improved tools for type IV effectors prediction, analysis and comparison in proteobacteria

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Abstract

Bacterial pathogens have evolved numerous strategies to corrupt, hijack or mimic cellular processes in order to survive and proliferate. Among those strategies, Type IV effectors (T4Es) are of increasing interest for basic research, including comprehension of hijacked cellular pathways, manipulated innate immunity, and application for therapeutics. To date, no other T4Es prediction tool is available online. Thus, scientists studying any symbiotic or pathogenic bacteria will benefit from powerful user-friendly tools to rapidly identify candidate T4Es and eventually disease targets of these effectors. Prediction of T4Es, especially for less-studied bacteria, must take into account archetypal effector characteristics such as eukaryotic-like domains or localization signals, and C-terminal features associated with type IV secretion. We present Searching Algorithm for Type IV Effector proteins (S4TE) 2.0, our web-based suite of tools for predicting and comparing repertoires of T4Es in user-specified genomic sequences. It represents a significant improvement over machine learning approaches that require sets of positive and negative controls). S4TE 2.0 is the only available T4Es prediction tool in which queries can be fully customized to work with any available bacterial genome. S4TE 2.0 also includes tools to analyze the genome architecture and the distribution of predicted T4Es depending on local gene density. S4TE 2.0 allows comparing candidate repertoires of T4Es in up to four strains. Using a scoring function S4TE 2.0 calculates the probability that a particular protein is a good match for a T4E. It is therefore uniquely capable of identifying unknown proteins as T4E with biologically relevant functions.

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The metalloenzyme ENO1 is ubiquitinated by *Ehrlichia chaffeensis* TRP120

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Abstract

Ehrlichia chaffeensis modulates numerous host cell processes, including gene transcription to promote infection of the mononuclear phagocyte. Modulation of these host cell processes is directed through the *E. chaffeensis* effectors, including TRP120. We previously reported TRP120 moonlights as a HECT E3 Ub ligase involved in ubiquitination of host cell transcription and fate regulators (PCGF5 and FBW7) which enhances infection. In this study we examined relationship between TRP120 and α -enolase (ENO1), a metalloenzyme that catalyzes glycolytic pathway substrate dehydration; however, alternative translation produces the c-myc promotor binding protein-1 (MBP-1) that functions as a transcriptional repressor. Notably, we recently demonstrated that knockdown of ENO1 enhanced *E. chaffeensis* infection, suggesting that ENO1 or MBP-1 may be degraded to promote infection. In order to investigate ENO1/MBP-1 as a potential TRP120 E3 ligase substrate, we first examined ENO1 protein level and the interaction between TRP120 and ENO1 in *E. chaffeensis*-infected cells using an ENO1-specific antibody. Immunoblot analysis demonstrated that levels of ENO1 were significantly decreased during *E. chaffeensis* infection. Immunofluorescence microscopy also revealed colocalization of ENO1 with TRP120 expressing *E. chaffeensis* morulae. To further demonstrate the interaction between TRP120 and ENO1, coimmunoprecipitation was performed confirming this interaction. We then examined whether TRP120 could ubiquitinate ENO1 using an *in vitro* ubiquitination assay and detected the generation of a higher molecular weight ENO1 isoform (~60 kD) in the presence of recombinant TRP120. Future directions include elucidation of the specific role of ENO1 ubiquitination by TRP120 during infection, and further expand knowledge regarding the important role of TRP120 Ub ligase function during infection.

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Sequencing protocol modification and validation for increased efficiency of *Rickettsia* species determination in clinical specimens

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Abstract

Rickettsia spp. are obligate intracellular gram-negative bacterium and the causative agents of rickettsial infections. These agents are classified into two subgroups, spotted fever group *Rickettsia* (SFGR) and typhus fever group *Rickettsia* (TFGR). Standard DNA sequencing protocols for SFGR and TFGR involves nested PCR, gel extraction, and sequencing. This current process is time consuming and increases background DNA, which may result in limited sequence quality and sensitivity. We modified then validated a streamlined process that is more rapid and sensitive than the standard procedure. Primer/probe sets from the original OmpA and 17kDa protocols were used. The optimization of a single PCR and a fast DNA separation and recovery system reduced amplicon preparation time from the traditional 8 hours to 3 hours with a cleaner product. Known concentrations of *R. typhi* and *R. rickettsii* DNAs were used to determine protocol sensitivity, resulting in limits of detection at 4 and 10 copies per reaction for 17kDa and OmpA, respectively. Assay verification was performed by testing a blind panel of DNA extracted from contrived blood specimens. Results correlate with expected values and show high reproducibility. Verification was performed with previously tested clinical specimens. Results demonstrate that the modified sequencing process can effectively identify *R. akari*, *R. felis*, *R. typhi* and *R. prowazekii* using the 17kDa target and *R. africae*, *R. conorii*, *R. parkeri* and *R. rickettsii* using the OmpA target in clinical samples. The modified methodology provides sensitive and specific results, therefore serving as an effective and more rapid process to determine *Rickettsia* species infection in clinical specimens.

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Development and validation of real-time PCR assays to detect *Rickettsia typhi* in clinical specimens

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Abstract

Rickettsia typhi (endemic or murine typhus) is an obligate intracellular gram-negative bacterium closely related to the category B biothreat agent, *R. prowazekii* (epidemic typhus). Endemic and epidemic typhus manifest similar clinical symptoms, including fever, headache, malaise, and rash. Laboratory confirmation of *R. typhi* infection involves serological methods such as IgG indirect immunofluorescence antibody assays (IFA). However, determination of the causative rickettsial species is not possible due to low specificity of this technique. IFAs also require an antibody response, which may not mount until ~2 weeks after onset. Thus, there is a need for rapid, more sensitive, and specific assays for acute samples in clinical labs. We developed and validated two real-time PCR assays for the detection of *R. typhi*, RT12 and RT27. Primer/probe concentrations were optimized and analytical specificity was tested using a panel consisting of *R. typhi* isolates, bacterial near neighbor DNAs, and environmental DNAs. Known concentrations of *R. typhi* DNAs were used to determine assay sensitivity for RT12 and RT27, resulting in limit of detections at 3.5 and 2.5 copies per reaction (efficiency = 100%), respectively. Assay verification was performed by testing a blind panel of DNA extracted from contrived specimens (blood), and results correlated with expected values. Repeat testing showed high reproducibility. Clinical verification was assessed with previously tested clinical specimens, demonstrating that the assays are capable of detecting *R. typhi* successfully. The two real-time PCR assays are sensitive and specific, serving as effective and rapid tools to detect *R. typhi* DNA in clinical specimens.

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Does it work? Effects of permethrin-treated uniforms on tick submissions to a passive tick surveillance program

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Abstract

Since 1989, the Army Public Health Center Tick-Borne Disease Laboratory (TBDL) has operated the Department of Defense Human Tick Test Kit Program (HTTKP), which receives human-biting ticks removed from military-affiliated personnel. These ticks are identified, assayed for human pathogenic agents, and the results are reported back to the tick bite victims and their physicians as actionable evidence for use in determining the appropriate treatment regimen. Among the data collected through the HTTKP, participants are asked if they were wearing permethrin-treated clothing at the time of the tick encounter. In 2012-2013, the permethrin-treated Army Combat Uniform was made available to Active Duty Soldiers, Army National Guard/Army Reserve Enlisted Soldiers, and the Senior/Junior Reserve Officers Training Corps (ROTC). Here we use the passive surveillance data collected through the HTTKP to determine the efficacy of permethrin-treated uniforms at protecting DoD personnel from tick bites and tick-borne diseases. We analyze whether fewer ticks were submitted by self-reported users of the permethrin-treated uniforms, whether certain cohorts of users are better protected than others, and whether certain tick species are better repelled than others.

39 -

The putative auto-transporter proteins ScaD and ScaE from *Orientia tsutsugamushi* are sufficient to mediate adherence to target mammalian cells

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Abstract

Orientia tsutsugamushi is the causative agent for scrub typhus, a vector-borne zoonotic disease of rising concern transmitted through the bite of a chigger. While this organism bears significant phylogenetic homology to other members of the family *Rickettsiaceae*, its pathogenesis remains less characterized than its genus *Rickettsia* counterparts. Recently, genomic analysis of *O. tsutsugamushi* identified several putative proteins bearing predicted structural homology to a family of *Rickettsia* auto-transporter proteins (surface cell antigens or Sca proteins), many of which have been implicated in bacterial pathogenesis. Interestingly, among the *O. tsutsugamushi* orthologs described, ScaC and ScaA have been demonstrated to function as bacterial adhesion factors, but the roles of the remaining orthologs have yet to be elucidated. Herein, we utilize a heterologous system to express *Orientia tsutsugamushi*, Ikeda strain, ScaD and ScaE at the surface of *E. coli* to investigate the roles of these proteins in pathogen-host cell interactions. Our results demonstrated that expression of ScaD or ScaE at the outer-membrane of *E. coli* (BL21(DE3)) is sufficient to mediate adherence to putative target non-phagocytic mammalian host cells. Whether any of these Sca protein homologues is also sufficient to mediate internalization into target host cells is currently under investigation. Our recent results *in vitro* indicate that interactions of *O. tsutsugamushi* with mammalian host cells is multifactorial and likely involves the function of several adhesin-receptor pairs to colonize a mammalian host.

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Identification pipeline of Anaplasmataceae type IV effectomes.

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Abstract

Anaplasmataceae family includes obligate intracellular pathogenic *Ehrlichia* and endosymbiotic *Wolbachia* bacteria. A key factor of bacterial pathogenesis and symbiosis with eukaryotic cells is the ability to evade the innate immune system and hijack the host cellular pathways. *Anaplasmataceae* use effector proteins (T4Es) to manipulate cellular processes in order to survive and proliferate. It is still difficult to predict and study the repertoires of T4Es in *Anaplasmataceae*. Identifying such type IV effectomes is crucial to comprehend how the bacterium establishes symbiosis or pathogenesis. Deciphering bacterial interactions with mammalian or vector cells will foster development of alternative strategies to fight against the pathogen or prevent pathogen transmission by the vector. We propose a pipeline to identify T4 effectomes in *Anaplasmataceae*. We first use S4TE 2.0 software as a prediction tool for T4Es. The predicted effectors are confirmed using secretion assays in *Legionella pneumophila* and with cellular biology approaches. Then, we screen the effectome library for intracellular localization, for particular cellular phenotypes, and for protein partners or chromatin interactions. We then investigate for potential post-translational modifications of effectors after secretion (phosphorylation, truncation). Finally, we do phenotypic screening after ectopic expression in yeast. For each remarkable phenotype, the corresponding effector genes is silenced using PNA technology. This computational based-medium-throughput screening of *Anaplasmataceae* type IV effectors will accelerate the dissection of bacteria-host mutualistic or pathogenic interactions and will highlight the evolutionary history shared by these bacteria. These results will promote the development of novel strategies to prevent vector-borne transmission of pathogens and alternative therapeutics.

36 -

Developing a recombinant *Coxiella* O-antigen

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Abstract

Coxiella burnetii is the causative agent of the devastating infectious disease Q fever. Q fever readily infects ruminant animals and can easily be transmitted to humans due to its low infectious dose. The bacterium displays a single surface exposed lipopolysaccharide (LPS) linked O-antigen. This is the best described virulence determinant of *C. burnetii*. As polysaccharides have an excellent track record as vaccines, the O-antigen would make an ideal component for a conjugate vaccine. However, it is very challenging to prepare O-antigen from *Coxiella*. We are therefore determining the biosynthetic pathway for the *C. burnetii* O-antigen. Our approach is to recombinantly produce and purify proposed O-antigen biosynthetic enzymes; and to develop a suite of enzymatic and biophysical assays to characterize these enzymes. These will facilitate reconstruction of the O-antigen pathway *in vitro*, and ultimately *in vivo*. In particular, we are reconstructing the biosynthesis pathway for virenose and dihydrohydroxystreptose, as these sugars are rarely found outside *C. burnetii*'s O-antigen. We are investigating the glycosyltransferases necessary for assembling the O-antigen from precursor sugars. Once we have identified all of the relevant enzymes, we will incorporate these into *E. coli* to provide a recombinant O-antigen for the *Coxiella* community.

34 -

Urine metabolite analysis of experimental animal with *Orientia tsutsugamushi* infection

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Abstract

Scrub typhus is an acute febrile mite-borne endemic disease in Asia-Pacific region. It is one of the representative seasonal disease which occurs in autumn. It was reported that 20.35 infected people out of 100,000 people in 2017 in Korea and its incidence has gradually been increased. To understand metabolites profiling after *Orientia tsutsugamushi* infection, balb/c mice were infected with lethal dose 100 (LD100) of *O. tsutsugamushi* Boryong, and the metabolic profiles were analysis with the urine of mice at final stages using liquid chromatography-(LC-) and flow injection analysis-tandem mass spectrometry (FIA-MS/MS). 65 metabolites whose concentration are statistically different were identified between experimental groups. Among them, acylcarnitines, glycerophospholipids, biogenic amines, and amino acids are major metabolite groups whose concentration significantly differ. Major perturbed metabolic pathways were tryptophan, glycerolipid, and phospholipid metabolism, suggesting that the nature of host responses are highly multi-factorial. In this study, we reported metabolite changing profiles in the mice infected with *O. tsutsugamushi*. This information will provide the cue for understanding the change of metabolic pathway after *O. tsutsugamushi* infection and will be valuable for further biomarker discovery in febrile diseases. This study was supported by a research grant (2018-NI002-01) of Korea Centers for Disease Control and Prevention.

Distribution of *Orientia tsutsugamushi* in *Leptotrombidium* mites, scrub typhus vector and reservoir
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Abstract

Leptotrombidium spp. are the major vectors for *Orientia tsutsugamushi*, an obligate intracellular Gram-negative bacterium, and causative agent of scrub typhus in human. The *Leptotrombidium* mites serve as disease vectors by horizontal transmission of bacteria to humans via the bite from larval mites, and represent true reservoir hosts with vertical transmission and maintenance of *Orientia* in nature. *O. tsutsugamushi* is maintained naturally by trans-stadial transmission (between life cycle stages) and trans-ovarial transmission (female adult to egg). However, little is known about the interactions and relationship between *Leptotrombidium* mites and *O. tsutsugamushi* during their life cycle. We developed chromogenic and fluorescence immunohistochemistry (IHC) techniques to observe the distribution of *O. tsutsugamushi* in all stages of *Leptotrombidium* mites. A 47 kDa *htrA* gene-based qPCR assay was used to quantitate *Orientia* in each life stage of *Leptotrombidium chiangraiensis*, *L. deliense*, and *L. imphalum*. The qPCR results suggested that *Orientia* load varied depending on the developmental stage of eggs, larvae, nymphs, and adults, indicating a positive correlation between the bacterial copies and the developmental stage of mite. The IHC showed systemic infection of *O. tsutsugamushi* in chiggers, nymphs, and female adults of all three species, but never in male adults. High density of *O. tsutsugamushi* organisms was observed especially in the brain (supraesophageal and subesophageal ganglia) and gastric caeca. Further investigations are required to understand the biological mechanisms for *O. tsutsugamushi* survival and transmission among *Leptotrombidium* mites, the pathogen-vector interactions, as well as novel chigger control strategies.

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Dynamics of the cytokine storm in experimental scrub typhus non-human primate model (Rhesus macaques) intradermally inoculated with *O. tsutsugamushi* Karp and Gilliam strain

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Abstract

Orientia tsutsugamushi is the causative agent of scrub typhus. The high genetic diversity of *Orientia* necessitates evaluation of the immunopathophysiology of different strains. Cytokine storm is associated with severe complications in scrub typhus. Understanding the cytokine response dynamics could reveal underlying mechanisms in immunopathogenesis. In this study, we compared bacteremia and multiple cytokine profiles in the rhesus macaque model using two different *O. tsutsugamushi* strains: Karp (highly human pathogenic strain) and Gilliam (Intermediate human pathogenic strain). Two groups of animals (n= 4) were intradermally inoculated with either Karp or Gilliam to the anterior thigh. Blood samples were collected on Days 0, 6, 12, 18, 28 and 80 post-inoculation. Multiplex cytokine profiles (23-cytokines) involved in immune regulation and bacteremia kinetics were compared. We found that the timing of cytokine elevations related to the bacteremia curve. Gilliam had an earlier onset of the bacteremia, between Day 3-12 (peaked: Day 6), compared to Karp between Day 6-15 (peaked: Day 12). In both groups, both pro-(IFN- γ , IL-15, IL-6, IL-18) and anti-(IL-1ra, IL-10) inflammatory cytokines reached the highest levels during bacteremia peaks. After the bacteremia (Day 18), TNF- α increased significantly in both groups. All cytokines returned to baseline levels by Day 28. Although infection doses of the Karp and Gilliam were different, cytokine profile patterns were similar. These results are evidence that scrub typhus is closely associated with cytokine-mediated immunopathogenesis, and could have an impact in the identification of immune biomarkers of disease status and correlates of protection in humans.

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The occurrence of *Anaplasma phagocytophilum* in cattle after grazing in the Republic of Korea

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Abstract

Anaplasma phagocytophilum is a zoonotic tick-borne intracellular bacterium causing tick-borne fever in domestic ruminants. In this study, we investigated the prevalence of *A. phagocytophilum* using specific PCR assays. Blood samplings were performed two times (before grazing; April and after grazing; November). On April, 39 blood samples were collected and on November, 39 blood samples were collected from the same cattle. The presence of *A. phagocytophilum* was examined by PCR and IFA. The 12 (15.4%, 12/78) blood samples were positive for *A. phagocytophilum*. However, serum samples were all negative by IFA. Interestingly, *A. phagocytophilum*-positive samples were found only in cattle after grazing. These cattle were negative before grazing. None of cattle exhibit any clinical manifestations. 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. This is the first study to report *A. phagocytophilum* infection in Holstein cattle in the ROK. This result suggests that cattle may play a role in reservoirs for *A. phagocytophilum* infection. 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.

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Nitric Oxide Reduces *Rickettsia rickettsii* Viability

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Abstract

Many cell types synthesize nitric oxide (NO) as part of their antimicrobial activity to limit the growth of microorganisms. Macrophages and endothelial cells infected with *Rickettsia conorii* or *Rickettsia prowazekii* require NO synthesis to control the replication of these intracellular, parasitic pathogens. However, the role of NO production in control of *Rickettsia rickettsii* (*R. rickettsii*) has not yet been examined and the molecular mechanisms by which NO exerts antimicrobial activity on *Rickettsia* spp. are unclear. Herein, we developed assays to examine the antimicrobial activity of NO on *R. rickettsii* in unstimulated endothelial cells and in cell-free media with NO-releasing polyamine compounds. In both models, NO reduced *R. rickettsii* viability; thus, NO is bacteriocidal to *R. rickettsii*. In cell-free media, *R. rickettsii* are more sensitive to NO in carbon-free phosphate buffer than in rich media. Indeed, *R. rickettsii* challenged with NO could not maintain ATP pools and ATP supplementation partially rescued *R. rickettsii* viability. Furthermore, supplementation with tricarboxylic acid cycle intermediates or amino acids increased the resistance of *R. rickettsii* to nitrosative stress compared to controls in carbon-free buffer. Together, these results indicate that NO is bacteriocidal to *R. rickettsii* and suggest that central metabolism is a target of NO in these bacteria. This work enhances our knowledge of anti-Rickettsial mechanisms of the innate immune system.

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Regulation of CX3CL1 expression in human microvascular endothelial cells during *Rickettsia rickettsii* infection by microRNA-424

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Abstract

Cytokines and chemokines trigger complex intracellular signaling through specific receptors to mediate immune cell recruitment/activation at the sites of infection. Fractalkine (CX3CL1) is a membrane-bound chemokine capable of facilitating intercellular interactions as an adhesion molecule and implicated in inflammatory insults by virtue of its chemoattractant functions. A published report (Valbuena and Walker, 2005) has documented increased CX3CL1 expression and a potential correlation with macrophage infiltration in target tissues in a murine model of spotted fever rickettsiosis. In this study, cultured human microvascular endothelial cells (HMECs) were infected with *Rickettsia rickettsii* for different time durations to determine CX3CL1 mRNA and protein expression by quantitative real-time PCR and Western blotting, respectively. Our results reveal 10.5 ± 2.5 -fold and 8.7 ± 2.1 -fold increase in CX3CL1 mRNA expression at 3h and 24h post-infection, coinciding with higher steady-state levels of the corresponding protein in comparison to uninfected HMECs. Since CX3CL1 is a validated target of microRNA (miR)-424 and our recent findings demonstrate robust down-regulation of miR-424 in HMECs infected with *R. rickettsii*, we next investigated the possibility of regulation of CX3CL1 expression during rickettsial infection by miR-424. As expected, *R. rickettsii* infection of HMECs resulted in $87 \pm 5\%$ reduction in miR-424 expression. Interestingly, a miR-424 mimic downregulated infection-induced expression of CX3CL1, whilst a miR-424 inhibitor yielded a reverse up-regulatory effect, suggesting miR-424-mediated regulation of fractalkine expression. Together, these findings provide the first evidence for the roles of host microRNAs in the regulation of an important chemokine implicated in innate immune responses to pathogenic rickettsiae.

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Genetic Heterogeneity of the *scaA-scaF* Autotransporter Genes of *Orientia tsutsugamushi*

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Abstract

Six autotransporter protein genes (called Surface Cell Antigens ScaA-F) are present in the genomes of *Orientia tsutsugamushi* (Ots). Specific TaqMan assays which amplify conserved regions of the beta barrel autotransporter domain of each of the six *sca* genes were previously used to determine the distribution of the genes among 178 isolate DNAs from 13 countries. In the present study, we extracted the *sca* gene sequences from the 54 available partial genome sequences of Ots. No new *sca* genes were identified and the distribution of genes was consistent with the TaqMan survey data (most isolate genomes had *scaA*, *scaC*, *scaD*, and *scaE* but many lacked *scaB* and/or *scaF*). We also conducted *in silico* and direct restriction fragment length polymorphism analysis (RFLP) of *scaC* and *scaE* passenger domains (PD) of the 54 genomes and 178 isolates, respectively. These two *sca* genes are the smallest of the six *sca* genes. The sizes of the PD amplicons for these two genes matched those obtained from the Ots genomic sequences (*scaE*: 1568-1589 bp, *scaC*: 839-866 bp). The bioinformatic analysis detected 34 distinct PD domains for both *scaC* and *scaE*. Four restriction enzymes cutting the PD domains were selected for RFLP. The four enzymes could distinguish 13-23 *scaC* and 23-26 *scaE* genomic PD types. Although a few new RFLP patterns and combinations were observed from the *in vitro* digestions of the isolate amplicons, the available *Orientia* PanGenome contains a good sampling of the *scaC* and *scaE* PD RFLP types present in our isolate repository.

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Characterization of an emergent large plaque variant of *Rickettsia rickettsii* Sheila Smith

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Abstract

Rickettsia rickettsii Sheila Smith is a highly virulent strain of *R. rickettsii* and is an etiological agent of Rocky Mountain spotted fever. While passaging *R. rickettsii* Sheila Smith through Vero cells, an emergent large plaque variant was observed and isolated. This variant produced plaques on Vero cell monolayers that were roughly three times larger than the expected size, with defined, rounded edges. Measuring the rate at which these *Rickettsia* strains successfully invade host cells revealed that Sheila Smith large-plaque (SS-L) can invade Vero cells at about twice the rate of Sheila Smith small-plaque (SS-S). However, analysis of growth curves suggest that these two Sheila Smith variants have the same rate of growth. Concurrent with the development of the large-plaque variant, a point deletion was discovered in *A1G_06515*, a gene that was previously identified as a potential gene of interest when Sheila Smith was compared to Iowa to identify genes that might be responsible for differences in virulence. This deletion results in a forty percent truncation of the encoded protein. The connection between this mutation and the observed phenotypes of the variant is currently being explored.

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Degradation of Tumor Suppressor FBW7 by *Ehrlichia chaffeensis* TRP120-Mediated Ubiquitination Promotes Infection

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Abstract

HME is an emerging tick-borne zoonosis caused by the obligately intracellular, gram-negative bacterium, *Ehrlichia chaffeensis*. Its survival is dependent on secreted tandem-repeat protein (TRP) effectors, which function to exploit evolutionarily conserved host cell proliferation and survival pathways Notch and Wnt for infection. We previously determined that *E. chaffeensis* E3 ligase TRP120 interacts with the human tumor suppressor E3 ligase FBW7. FBW7 negatively regulates a network of oncoproteins (Notch, c-Jun, MCL1 and cMYC) that play central roles in cell division, growth, differentiation and survival. Thus, we hypothesize that *E. chaffeensis* TRP120 ubiquitinates host tumor suppressor FBW7 for degradation to stabilize Notch signaling pathway and other oncoproteins to promote infection and intracellular survival. In this study, we demonstrate TRP120 and FBW7 interaction through immunofluorescent microscopy colocalization and co-immunoprecipitation and determined that FBOX and WD40 domains in FBW7 interact with TRP120. During *E. chaffeensis* infection, although FBW7 gene expression increased, protein levels decreased dramatically. Moreover, a reduction in FBW7 coincided with increased levels of Notch, MCL-1, phosphorylated c-Jun and cMYC, which are negatively regulated by SCF^{FBW7} ligase activity. An increase in FBW7 K48-ubiquitination was detected during infection by co-IP, and FBW7 degradation was inhibited in infected cells treated with the proteasomal inhibitor bortezomib. Direct ubiquitination of FBW7 by TRP120 was demonstrated *in vitro* and confirmed by ectopic expression of TRP120 catalytic mutant. These results suggest that TRP120 ligase activity promotes ehrlichial infection by degrading FBW7 to maintain stability of Notch and other oncoproteins involved in cell survival and apoptosis.

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The identification of *Coxiella burnetii* virulence-associated genes in *Galleria mellonella*

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Abstract

Coxiella burnetii is an intracellular pathogen responsible for causing Q fever in humans, a disease with varied presentations ranging from an acute flu-like sickness to a chronic illness that can result in endocarditis. *C. burnetii* is classified as a category B bio-threat by the CDC, and current treatment is limited to extended periods of antibiotics, with no vaccine available in the UK at present. Together, this presents a worrying situation which calls for improved antibiotic therapies and/or the production of a safe, effective vaccine. The larvae of the greater wax moth, *Galleria mellonella* are an excellent model for the investigation of bacteria, and are being increasingly used to test antibiotic efficacy and to identify virulence mechanisms. The aim of this project was to develop *G.mellonella* models for *C.burnetii* infection, including a model with increased sensitivity. We have produced a reliable, reproducible screen using *G.mellonella* for the investigation of *C.burnetii*. Challenging larvae with 10⁶GE/mL of *C.burnetii*, we obtain 100% mortality within an 8 day timeframe, permitting the identification of attenuated phenotypes. In addition, we have increased the sensitivity of the model through pre-treatment of *G.mellonella* with the steroid drug dexamethasone. By pre-treating larvae with 200µg dexamethasone 21-phosphate, a significant increase in mortality is observed (p=0.0002). The ability to identify attenuation has been confirmed by testing a weakly attenuated NMII::Tn-RelA mutant, which shows a significant delay in mortality (p=0.01). These two models will now enable the screening of transposon mutants to robustly determine attenuated phenotypes, allowing the identification of virulence-associated genes.

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Elucidating and exploiting O-antigen biosynthesis for Q fever vaccine development

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Abstract

Coxiella burnetii is an obligate pathogen of ruminants, with a worldwide distribution. Miscarriages caused by the resulting “Q fever” not only have economic repercussions for farmers, but also deposit huge amounts of bacteria back into the environment. Here, by adopting a spore-like state, *C. burnetii* can survive and infect new hosts, including humans. Human infections generally present with flu-like symptoms, however, rare cases can develop chronic Q fever with complications such as chronic fatigue, heart valve infection, or endocarditis. There is no licensed Q fever vaccine in the UK/EU/US. The lipopolysaccharide (LPS) of *C. burnetii* is the target for current vaccine development as this provides the main determinant of virulence. The proposed pathways for LPS-linked O-antigen biosynthesis are being tested with recombinantly expressed *Coxiella* ORFs and enzymes from paralogous sugar biosynthetic pathways in *Escherichia coli*. In particular, comparison to *E. coli* dTDP-rhamnose biosynthesis is providing clues to the biosynthesis of dihydrohydroxystreptose (DHHS), a sugar thought to be unique to *C. burnetii*’s O-antigen. Elucidating the pathways to biosynthesis of *Coxiella*’s unusual O-antigen sugars provides the basis for generating a recombinant O-antigen fragment for use in a glycoconjugate vaccine.

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Vaccination with recombinant Asp14 and OmpA of *Anaplasma phagocytophilum* in lambs gave serological responses, but ineffective protection against challenge.

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Abstract

Anaplasma phagocytophilum is the most wide spread tick borne pathogen in farm animals in Europe and is known as the agent of tick borne fever in sheep. Lambs are especially susceptible to the infection, which leads to immune suppression and secondary infections such as septicemia, pyemia, arthritis and pneumonia. Vaccines are currently not available. Recent studies in cell cultures and mice have shown promising results of the infection-blocking effect by antibodies against the Asp14 and OmpA membrane proteins of *A. phagocytophilum*. However, studies in heifers, using the *Anaplasma marginale* analogues of the proteins did not produce protective immunity against challenge. The current study used recombinant Asp14 and OmpA as a vaccine in lambs as two injections, 21 days apart. Five lambs were vaccinated with each protein and five lambs were kept as unvaccinated controls. Experimental challenge with a wild isolate of *A. phagocytophilum* (Gen.bank M73220) was performed in vaccinated and controls, 21 days after the last vaccination and the animals were monitored for two further weeks. Lambs in both vaccinated groups responded with serum antibodies against OmpA and Asp14, detected from day 28 after the first vaccination. After challenge, both vaccinated and controls developed bacteremia and clinical signs consistent with *A. phagocytophilum* infection. Cellular immune responses showed similar patterns across the groups, which indicate lack of specific cellular responses to the vaccines. In conclusion, the analyses of serological, cellular and clinical responses revealed no protective effects of recombinant OmpA and Asp14 in lambs against challenge with *A. phagocytophilum*.

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Molecular Detection of *Anaplasma* spp. in *Xenarthra* in Brazil

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Abstract

The *Xenarthra* Order comprises armadillos, sloths and anteater, which are distributed from the south center of North America to the south of South America. The present study aimed to investigate the occurrence of *Anaplasma* spp. free-living mammals of the *Xenarthra* order from four states in Brazil. Blood samples were collected from sloths rescued during a powerplant flooding in the states of Rondônia and Pará. Spleen fragments from armadillos and anteaters hit by vehicles on highways in Mato Grosso do Sul, and from animals necropsied at the Wildlife Pathology Service at the Faculty of Agricultural and Veterinary Sciences of Jaboticabal-SP, totalizing 336 animals (195 brown-throated sloths (*Bradypus variegatus*), 3 *Bradypus* sp., 4 two-toed sloths (*Choloepus didactylus*), 31 *Choloepus* sp., 30 southern tamandua (*Tamandua tetradactyla*), 50 giant anteaters (*Myrmecophaga tridactyla*), 3 Southern naked-tailed armadillos (*Cabassous unicinctus*), 11 nine-banded armadillos (*Dasypus novemcinctus*), 8 six-banded armadillos (*Euphractus sexcinctus*) and 1 giant armadillo (*Priodontes maximus*)). The results showed that 20,24% (68/336) of the samples were positive in a conventional PCR for Anaplasmataceae based on the *rrs* gene. Out of these samples, 57.35% were shown to be positive in a nested PCR for *Anaplasma* spp. based on the *rrs* gene. Seven samples (17.95%) out of nPCR-positive samples were also positive in the qPCR for *A. phagocytophilum* based on the *msp-2* gene. The *rrs* obtained sequences were phylogenetically associated with genotypes of *Anaplasma* spp. obtained from rodents in Brazil, *A. ovis* and *A. phagocytophilum*. The present study demonstrates the occurrence of *Anaplasma* spp. in mammals of the order *Xenarthra* in Brazil.

19 -

Eukaryotic protein mimicry as an infection strategy for *Ehrlichia chaffeensis*

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Abstract

Ehrlichia chaffeensis, the causative agent of the life-threatening zoonosis human monocytic ehrlichiosis, is an obligately intracellular, gram-negative bacterium that primarily infects monocytes. *E. ch.* multifunctional effector and surface protein TRP120 has been implicated as an adhesin and invasin and has been shown to be sufficient for induction of phagocytosis in monocytes, but a cognate host receptor remains to be identified. Our lab recently published that *E. ch.* activates host monocyte Wnt signaling early during infection to promote bacterial uptake and intracellular survival, and that activation is mediated by TRP120. Wnt signaling is a conserved eukaryotic signal cascade initiated by the binding of a Wnt ligand to a Frizzled (Fzd) receptor that regulates cellular events including innate immunity and phagocytosis. We hypothesize that TRP120 is a novel Wnt signaling ligand mimic that binds Fzd receptors to stimulate bacterial phagocytosis and Wnt pathway activity seen during infection. In this study, we demonstrate that RNA silencing of the Wnt receptor complex significantly reduces *E. ch.* infection in THP-1 monocytes, and that *E. ch.* colocalizes with multiple Fzd homologues early during infection. We use *in silico* approaches to predict both sequence and functional similarity between TRP120 and various Wnt ligands and show a sequence within the TRP120 tandem repeat domain induces Wnt pathway activity. Finally, we demonstrate direct binding between TRP120 and multiple Fzd proteins. These findings elucidate a mechanism of host cell entry that links TRP120 adhesin activity and ehrlichial activation of Wnt signaling, and they identify a potential target for antimicrobial therapeutics.

18 -

Development of *E. chaffeensis* TRP antigen detection assay for early diagnosis of human monocytotropic ehrlichiosis

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Abstract

Human monocytotropic ehrlichiosis (HME) is a tick-borne emerging disease and one of the most prevalent life-threatening zoonoses in the United States. HME results in patient hospitalization in 50-70% of cases and has a fatality rate of 3% due to inaccurate diagnosis and delays in treatment. HME is caused by an obligately intracellular bacterium, *Ehrlichia chaffeensis* (*E.ch*). Clinical diagnosis of HME is usually confirmed by detection of *Ehrlichia*-specific antibodies in patient sera using an indirect fluorescent antibody assay (IFA). Currently there are no point-of-care (POC) antigen detection tests for HME diagnosis. Our laboratory has characterized secreted effector proteins including TRP120, TRP47 and TRP32 and reported that they are expressed at high levels during infection. Hence, TRPs are likely circulating in the peripheral blood and potential diagnostic biomarkers. In this study, we developed and optimized a colorimetric sandwich antigen capture ELISA. A total of 19 suspected HME patient sera were tested for the presence of TRP32 and TRP120 antigens, and 10 samples were positive for both TRP32 and TRP120. Sensitivity of the ELISA was in the nanogram range (≤ 40 pg). When coupled with ultrasensitive detection approaches, we anticipate that an assay that provides rapid, sensitive and specific detection of *E. chaffeensis* TRPs can be developed for POC and reference laboratory settings.

17 -

Quantitative analysis of *Rickettsia rickettsii* in salivary glands of adult *Dermacentor variabilis* during the initial period of feeding

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Abstract

Our previous studies demonstrated that *Rickettsia rickettsii* is present in salivary glands [SG] of unfed ticks and is inoculated into the skin of model animals with the very first portions of saliva. Additional amounts of the pathogen are introduced as feeding continues resulting in increasing severity of infection in the host. This suggests propagation of *Rickettsia* in SG during feeding. This study assessed the dynamics of *R. rickettsii* in SG of infected *D. variabilis* through the crucial first 48 hours of tick feeding. Attached ticks were removed from hosts at 4-8 h intervals from 0 to 48 h post-attachment. Quantities of rickettsial DNA in SG were quantified using qPCR and normalized to the amount of tick DNA in each sample. For comparison, the quantity of rickettsiae in haemolymph of the same individual ticks was assessed by testing 2 amputated legs per tick. The rickettsial copy numbers in either sample type ranged from 10^2 to 10^7 precluding identification of statistically significant differences between any time points. However, the trend of the mean demonstrates that the quantity of *R. rickettsii* in SG of feeding ticks does not increase continuously through the first 48 h of feeding, but oscillates up and down at 12-18 h intervals presumably reflecting the balance between processes of rickettsial proliferation and evacuation with saliva. Dynamics of rickettsiae in SG did not correlate with those in the haemolymph indicating that quantitative changes are due to rickettsial proliferation within the salivary tissue itself rather than inflow of infected hemocytes.

15 -

Investigating the presence of *Rickettsia* spp. and *Yersinia pestis* in flea from the natural plague foci of Kazakhstan.

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Abstract

Kazakhstan possesses many active natural plague foci in the southern and western part of the Republic. In comparison with the plague foci, the presence of rickettsial pathogens in Kazakhstan is poorly studied. Moreover, the prevalence of rickettsia species that circulate in these regions is poorly understood. This project was focused on the possible co-infection of *Y. pestis* and *Rickettsia* spp. in a single vector (flea) mainly in the habitat of the large gerbil (*Rhombomys opimus*), the main host of the plague microbe. A total of 14,650 fleas were captured by five anti-plague stations. To date 732 pools containing the DNA of 7,491 fleas have been tested for *Y. pestis* and *Rickettsia* spp. Of these pools 263 were positive for *Rickettsia* DNA with the following results per APS. Kyzylorda APS – 155 pools (1572 fleas) with 15 (9.7%) positive (155/1572/15/9.7%); Aral APS – 153/1600/15/9.8%; Shymkent APS – 138/1399/67/48.5%; Zhambyl APS – 214/2200/136/63.5%; Uralsk APS – 72/720/3/4.2%. No DNA of *Y. pestis* was detected in flea samples. The results of the screening demonstrated the presence of at least one pathogen in the fleas obtained from the plague foci in 5 studied areas. It is worth mentioning the high rate of fleas' infection with rickettsiae in Zhambyl and Turkestan regions (South Kazakhstan oblast) - 63.5% and 48.5% of the pools. We expected to find *Y. pestis* samples in fleas captured in plague endemic areas. Negative results indicated the absence of active plague transmission in these regions. The work is ongoing to determine the rickettsiae species found in positive fleas.

10 -

***Ehrlichia chaffeensis* activation of Notch signaling increases XIAP expression to inhibit intrinsic apoptosis**

LaNisha Patterson, Jennifer Wang, Jere McBride

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Abstract

Human monocytotropic ehrlichiosis is a tick-borne zoonosis caused by the intracellular gram-negative bacterium, *Ehrlichia chaffeensis*. Studies have shown that *E. chaffeensis* suppresses apoptosis to promote host cell survival. The intrinsic apoptotic pathway is known to result in mitochondrial permeability transition, leading to the execution of apoptosis. During *E. chaffeensis* infection, mitochondrial potential is maintained, and modulation of intrinsic apoptotic regulators occurs. Our laboratory has shown host-pathogen interactions to occur through the tandem repeat protein effector, TRP120, which activates Notch signaling. Notch activation is known to play significant roles in functions, including innate immune mechanisms such as autophagy and apoptosis. Although Notch activation by *E. chaffeensis* has been shown to downregulate TLR expression, the role of Notch signaling in *E. chaffeensis* is unknown. Activation of Notch assists in inhibition of apoptosis by stabilizing expression of an anti-apoptotic protein, XIAP. Caspases cleave XIAP into two fragments; BIR1-2 and BIR3-RING, leading to differential inhibition of extrinsic and intrinsic apoptotic pathways. BIR3-RING fragments are potent inhibitors of intrinsic apoptosis. Thus, we hypothesize that TRP120 Notch activation stabilizes XIAP expression to inhibit intrinsic, caspase-dependent apoptosis. We investigated expression levels of XIAP during infection by collecting THP-1 cells infected with *E. chaffeensis*. Results demonstrated an increase in XIAP expression during infection. Importantly, increased XIAP expression was abrogated in Notch-1 knockout cells. Interestingly, cleavage of XIAP into the BIR3-RING domain occurred during later timepoints of infection. These results indicate that TRP120 Notch activation leads to XIAP stability to assist in inhibition of intrinsic apoptosis during *E. chaffeensis* infection.

8 -

Development of *Orientia tsutsugamushi* ScaA antigen-expressing recombinant non-replicative adenovirus virus vector for evaluation of protection against scrub typhus infection

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Abstract

Orientia tsutsugamushi is the etiologic agent of scrub typhus, which is the most prevalent rickettsiosis worldwide. *Orientia* is maintained transovarially by its reservoir hosts, trombiculid mites, which emerge as larvae from the soil seeking a meal. Humans develop an eschar at the site of inoculation, and subsequently the bacteria disseminate hematogenously throughout the body with infection of endothelial cells and macrophages. This study aims to develop *Orientia* ScaA antigen-expressing recombinant non-replicative adenovirus vector for evaluation of protection against scrub typhus infection with *O. tsutsugamushi*. ScaA has been found to stimulate protective immunity against lethal challenge of *O. tsutsugamushi*. A replication-deficient recombinant human adenovirus 5 expressing ScaA of *O. tsutsugamushi* Karp strain was prepared using In-Fusion technology. The entire ORF of *scaA*, a 4455 basepair fragment, was amplified by PCR using custom oligonucleotides containing 15 basepair extensions homologous to the end of the vector for efficient cloning of long inserts. This fragment was directionally cloned into pAdenoX-ZsGreen-1, an adenoviral expression vector designed to constitutively express a gene of interest and ZsGreen 1 (green fluorescent protein from *Zoanthus* sp.) in mammalian cells. Positive clones containing the *scaA* fragment were verified by DNA sequencing, and large-scale recombinant adenoviral plasmid DNA was purified. After linearization, transfection into human embryonic kidney cells (Adeno-X-293) was performed to amplify and purify the recombinant adenoviral particles. After PCR confirmation that the encapsidated adenoviral genome contained a functional copy of the sequence of interest, propagation of the recombinant adenovirus was performed to prepare high-titer stocks which will be used to immunize mice.

7 -

Distinct developmental stages during the intracellular life cycle of *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi is a mite-borne rickettsial bacterium that causes the life-threatening human disease scrub typhus. This disease is endemic in many parts of the Asia-Pacific and is estimated to affect over a million people annually. *O. tsutsugamushi* is an obligate intracellular bacterium, which escapes to the cytosol shortly after infecting host cells, then traffics to the perinuclear region for growth and replication prior to host cell exit. Here we have addressed the question: how do *O. tsutsugamushi* cells change over the course of this 5-7 day intracellular infection cycle? In order to address this question, we have used a combination of immunofluorescence microscopy, gene expression analysis and analysis of the bacterial metabolic activity of *O. tsutsugamushi* at different times after infection. This has led us to a model for the differentiation of *O. tsutsugamushi* into five distinct stages, and this will provide a useful framework for future studies on the cell biology of this organism.

6 -

The importance of iron in *Coxiella burnetii* replication, viability and virulence

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Abstract

Coxiella burnetii, the etiological agent of Query (Q) fever in humans, is a highly infectious obligate intracellular bacterium capable of causing both hepatitis and endocarditis. Following infection, *C. burnetii* replicates exclusively within a host-derived compartment known as the *Coxiella* Containing Vacuole. In natural infections, *C. burnetii* colonizes organs with functions in iron storage and recycling, suggesting pathogen physiology is tied to host iron metabolism. Iron was previously reported to have a limited role in *C. burnetii* virulence regulation despite evidence that host cells infected with *C. burnetii* increase transferrin receptor expression, suggesting active iron acquisition by the bacterium occurs upon infection. While the *C. burnetii* genome sequence indicates limited capacity to acquire iron via siderophores or uptake systems for iron-containing molecules, the pathogen does encode the ferrous iron transporter FeoAB suggesting that molecular iron is the natural iron source for *C. burnetii*. Through the use of axenic (host cell-free) media, the role iron plays in *C. burnetii* replication and therefore virulence was examined. We show that *C. burnetii* requires iron in excess of 5 μ M to replicate under axenic conditions and loses viability within 3 days post iron starvation. *C. burnetii* appears unique in its iron dependency during axenic growth compared to other Gram-negative bacteria, suggesting the bacterium is a ferriphile (i.e., has a fondness for iron). Additionally, we show that iron initiates *C. burnetii* protein synthesis and replication; and that the bacterium can utilize biologically diverse forms of iron. Thus, iron is essential for *C. burnetii* replication, viability and therefore virulence.

2 -

Characterization of a *Bartonella quintana* effector protein

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Abstract

Bartonella quintana is a Gram-negative, louse-borne, bacterial pathogen, and causes the disease trench fever. Trench fever was first described during the first World War, when it was estimated to have affected over one million people. Urban trench fever has since re-emerged among homeless, alcoholic, and displaced individuals. *B. quintana* can evade and suppress its host's immune system, allowing the bacteria to establish intra-erythrocytic infection and persist for months or years. *B. quintana* has a Type IV Secretion System which is important in establishing infection, though its effectors and their functions are poorly understood. One potential Type IV secreted effector is homologous to the *Yersinia* Type III secreted effector YopJ. *Yersinia* YopJ is an acetyltransferase that inhibits the MAPK and NF- κ B pathways in innate immune cells. The cellular target of the *B. quintana* YopJ homologue (Bq-YopJ) and its role in virulence are unknown. The aim of this project is to characterize the function of *B. quintana* YopJ in altering host cell signalling and to determine the mechanism by which it acts. We have performed protein interaction assays, including a yeast two-hybrid screen, to determine the host cell target protein of Bq-YopJ. Our preliminary results show that human cells transfected with Bq-YopJ have impaired signalling. We will present our results on the effect of Bq-YopJ on immune signalling pathways.

Systematic Review of Scrub Typhus Study Landscape: Protocol and Preliminary Literature Search Results

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Abstract

One billion people are at risk of scrub typhus. However, compared to its magnitude, evidence to optimise treatments and disease control are sparse. Existing data collected from past clinical trials and longitudinal observational studies could be a source of information to address research priorities and knowledge gaps. This review aims to conduct a systematic review to assess the characteristics of scrub typhus clinical studies and explore the feasibility to develop a scrub typhus individual participant-level data (IPD) platform. Six databases and two clinical trial registries were searched for clinical trials and longitudinal observational studies conducted between 1998 and March 2018. Variables for extraction include treatment tested, patient characteristics, diagnostic methods, geographical location, outcome measures, and statistical methodology. The literature searches identified 5,163 citations, of which 2,647 unique articles were independently screened by two reviewers. A total of 95 studies (7 clinical trials and 88 observational studies) met the pre-specified inclusion criteria. The studies have been conducted in 11 countries and enrolled a total of 9,010 patients. 390 case series and reports were also identified. Although there were only a few scrub typhus clinical trials found, there are substantially more data available from observational studies. Meta-analysis using an IPD approach can produce a more representative secondary analysis because it facilitates the use of data from observational studies as well. Understanding the landscape of scrub typhus treatment studies allows assessment of the feasibility of addressing research questions using IPD meta-analysis method and to conduct a research gap analysis.

Session 10: Pathogenesis I: *Coxiella*, *Orientia* and Ticks

Chair: Jason Carlyon & Mebratu Bitew

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The *Coxiella burnetii* Type 4B Secretion System blocks host endosomal maturation

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Abstract

Following host cell uptake, the *Coxiella*-Containing Vacuole (CCV) progresses through the endosomal maturation pathway to a phagolysosome with characteristics such as acidic pH, active proteases, and lysosomal markers. Approximately 24-48 hours post infection, heterotypic fusion between the CCV and host endosomes/lysosomes leads to CCV expansion and subsequent bacterial replication in the mature CCV. Formation of the mature CCV requires effector proteins secreted by the *Coxiella* Type 4B Secretion System (T4BSS), and activation of *Coxiella* metabolism and the T4BSS requires initial CCV acidification. However, we recently found that the mature CCV is more alkaline (pH~5.2) than lysosomes (pH~4.8) and that CCV acidification to pH~4.8 degrades *Coxiella*, suggesting *Coxiella* actively regulates CCV pH. Because heterotypic fusion with host endosomes/lysosomes may influence CCV pH, we investigated endosomal maturation in cells infected with wildtype (WT) or T4BSS mutant (Δ T4BSS) *Coxiella*. We observed significantly fewer LAMP1-positive lysosomes, along with more alkaline vesicles (pH~5.8), in WT-infected cells compared to mock or T4BSS-infected cells. Further, while endosomes progressively acidified from the periphery (pH~5.4) to the perinuclear area (pH~4.7) in both mock and Δ T4BSS-infected cells, endosomes did not acidify beyond pH~5.4 in WT-infected cells, suggesting that the *Coxiella* T4BSS inhibits endosomal maturation. Finally, increasing cellular lysosomal content by overexpressing the transcription factor EB inhibited *Coxiella* growth, indicating lysosomes are detrimental to *Coxiella*. Overall, our data suggest that *Coxiella* regulates CCV pH, possibly by reducing the number of host lysosomes available for heterotypic fusion.

22 -

Hypoxia-induced citrate limitation results in *C. burnetii* containment in macrophages

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Abstract

Coxiella burnetii is the causative agent of the zoonotic disease Q fever. Usually, humans get infected through the inhalation of contaminated aerosols. Alveolar macrophages are the first line of defence against inhaled *C. burnetii*. Apart from acute Q fever, around 2-5% of *C. burnetii* infected humans will develop chronic Q fever, which mainly manifests as endocarditis years after exposure. Details about how *C. burnetii* escape the immune system and persist for years inside the host are not fully established. Here we analysed how oxygen-availability influence the interaction of macrophages with *C. burnetii*. Thus, we infected murine bone marrow-derived macrophages (BMDM) with *C. burnetii* under normoxia (21% oxygen) or hypoxia (0.5% oxygen). Our experiments revealed that under normoxic conditions, *C. burnetii* replicates in BMDM and fails to induce robust accumulation of hypoxia-inducible factor 1 α (HIF1 α). Exposure to hypoxia, in contrast, induced stabilization of HIF1 α , which was further augmented upon infection. Our data indicated that stabilized HIF1 α is essential for inhibiting *C. burnetii* replication and induction of *C. burnetii* persistence. Mechanistically, HIF1 α impairs the activity of STAT3, which reduces the intracellular citrate level and consequently prevents *C. burnetii* replication. Interestingly, replenishing hypoxic macrophages with trimethyl citrate allowed *C. burnetii* growth. This suggests that persistence of *C. burnetii* in hypoxic macrophages is driven by oxygen-dependent citrate limitation. Our data suggest that regulation of citrate levels by HIF1 α represents a novel principle of nutritional pathogen-containment of *C. burnetii*, which might lead to a state of persistence and, thus, to chronic Q fever.

9 -

The *Coxiella burnetii* sterol modifying enzyme CBU1206 is critical for intracellular growth

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Abstract

Coxiella burnetii, the causative agent of Q fever, is a highly infectious obligate intracellular bacterium spread through aerosols. Inside the host cell, *Coxiella* replicates in a phagolysosome-like parasitophorous vacuole (PV) that appears to be sterol-rich. While dogma in the field dictated that cholesterol was essential for *Coxiella* growth, we recently discovered that *Coxiella* is exquisitely sensitive to PV cholesterol levels. Specifically, cholesterol accumulation in the *Coxiella* PV membrane reduces PV fusion and acidifies the PV lumen, resulting in bacterial death. Because cholesterol readily traffics to the PV, we hypothesize that *Coxiella* regulates PV cholesterol levels. We previously demonstrated that a *Coxiella* eukaryotic-like sterol reductase, CBU1206, is an active enzyme that can modify eukaryotic sterols and therefore may lower cholesterol in the *Coxiella* PV membrane through enzymatic modification. To further assess the role of CBU1206 during *Coxiella* infection, we generated a CBU1206 knockout (1206KO). While growth in broth media is not affected compared to wild type (WT) bacteria, the 1206KO displayed smaller PVs and a significant growth defect in both epithelial cells and macrophages. In contrast, 1206KO growth and PV size were normal in cholesterol-free cells. Cholesterol supplementation revealed that the 1206KO is hypersensitive to cholesterol, further suggesting a link between CBU1206 and host cholesterol. Compared to WT, 1206KO PVs accumulate sterols, are less fusogenic, and are more acidic. Collectively, these data suggest that CBU1206 plays an important role in regulating cholesterol levels in the *Coxiella* intracellular niche.

12 -

The function of ATGs in tick *Ixodes scapularis* autophagy in response to amino acid starvation

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Abstract

Ticks are obligate hematophagous arthropods and can tolerate starvation during off-host periods. Macroautophagy (hereafter autophagy) is a well-conserved self-eating mechanism of cell survival and essential for recycling cellular contents during periods of starvation, stress, and injury. Although the genome sequence of *Ixodes scapularis* (Say) is available, the characteristics and functions of autophagy-related genes (ATG) superfamily remain largely unknown. To advance our understanding of autophagy in *I. scapularis*, we utilized a comprehensive genomics approach to identify ATGs. To date, homologues of 14 ATG genes were identified, and their motif compositions were also predicted. Expression patterns of ATG genes differed across tick developmental stages. The Lysotracker staining and western blotting results indicated autophagy activation after amino acid starvation treatments in *I. scapularis* embryo-derived cell line ISE6. IDE8 cells will be tested as well for comparison. For ATG8 family, *Is-ATG8A* was activated significantly and *Is-ATG8C* decreased in both cell lines after amino acid starvation treatments. But the expression levels of *Is-ATG8B* showed no significant changes between the treated and control cells. We predict that silencing *Is-ATG8A* or *Is-ATG8C* will reduce starvation-triggered autophagy in ISE6 and IDE8 cells. All results demonstrated that ATG8 family members (ATG8A and ATG8C) play a key role in starvation-triggered autophagy in *Ixodes scapularis* tick. And our observations highlighted the molecular evolutionary properties and the response mechanism to amino acid deprivation of ATG genes in ticks.

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Biochemical Characterization of a Deubiquitinase Effector Protein from *Orientia tsutsugamushi*

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Abstract

Pathogenic bacteria often modulate the host ubiquitin proteasome system to hinder detection and destruction. Through computational screening we identified a putative deubiquitinase (DUB) domain containing protein from *Orientia tsutsugamushi*, which we found capable of cleaving K63- and K48-extended ubiquitin chains. In *cis* to the DUB domain we discovered a ubiquitin binding domain (UBD) that modulates DUB activity. Overexpression in yeast proved to be toxic, including fragments devoid of the DUB and *cis*-UBD domains. HeLa cell lysate pulldowns paired with mass spectrometry produced candidates which included: the clathrin adaptor complexes AP-1/AP-2 as well as the RhoGTPases Rac1 and CDC42. These were validated by co-immunoprecipitation from ectopically expressing HeLa cells and *in vitro* direct binding assays. The DUB protein binds AP-1/AP-2 through mapped dileucine motifs. The RhoGTPase binding is due to the presence of a validated Rac1/CDC42 guanine nucleotide exchange factor. After generating antibodies, we validated the expression and secretion of the effector during *O. tsutsugamushi* infection. Co-immunoprecipitation revealed the effector preferentially interacts with AP-2 during infection. Given the role of AP-2 in clathrin-mediated endocytosis we examined transferrin uptake in ectopically expressing HeLa cells and see a decrease in endocytic activity. Neither the AP-1/2 binding domain nor the GEF domain are responsible for the endocytosis defect. Instead, the defect is linked to a domain capable of binding membranes directly via a specific phospholipid. Cumulatively, the protein likely activates endocytosis and we are currently testing the role of the effector in bacterial entry.

Session 11: Pathogenesis II: *Ehrlichias* and *Rickettsias*

Chair: Stacey Gilk & Patrik Engstrom

11 -

***Ehrlichia chaffeensis* TRP120 Interacts with Notch Epidermal Growth Factor Domains to Activate Notch Signaling**

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Abstract

Ehrlichia chaffeensis is an obligately, intracellular gram-negative bacterium, and the etiological agent of human monocytotropic ehrlichiosis (HME); a life-threatening emerging tick-borne zoonosis. Recently, we have shown that the mechanisms whereby *E. chaffeensis* evades host defenses of the macrophage appear to involve activation of Wnt and Notch signaling. Various molecular-based strategies are used by *E. chaffeensis* for intracellular survival, including host-pathogen interactions by secreted tandem repeat protein effectors. TRP120 has been shown to interact with proteins associated with conserved signaling pathways Wnt, Notch and Sonic Hedgehog. We have demonstrated direct interaction of TRP120 with Notch proteins ADAM17 and FBW7. Further, colocalization of TRP120 with ADAM17 and the Notch-1 receptor has been demonstrated. Importantly, the defined ligand binding domain of Notch-1 are epidermal growth factor-like repeats (EGFs) 11-13 in the extracellular domain (NECD) of the receptor. Thus, we hypothesize that *E. chaffeensis* TRP120 is a novel Notch ligand that activates Notch signaling via TRP120-TR molecularly interacting with NECD-EGFs 11-13. We have demonstrated homology of TRP120 and Notch ligands. Specifically, a short motif in TRP120-TR, EDDT, shares homology with Notch ligands. Using surface plasmon resonance, we have determined direct binding of TRP120 and Notch-1 using a Notch-1 recombinant protein containing EGFs 1-13. We have further demonstrated that TRP120-TR and TRP120-NTD are responsible for Notch activation. Interestingly, recombinant TRP120-NTD contained the partial repeat EDDT. These results indicate TRP120 functions as a eukaryotic ligand mimic to activate Notch signaling. The current findings may allow for the development of novel therapeutics by targeting host-pathogen interactions.

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A biosafety level-2 dose-dependent lethal mouse model of spotted fever rickettsiosis

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Abstract

Rickettsia parkeri has been reported across the American continents associated with a relatively mild human disease characterized by eschar formation at the tick feeding site, regional lymphadenopathy, fever, myalgia and rash. Currently, there are several mouse models that provide good approaches to study the acute lethal disease caused by *Rickettsia*, but these models can only be performed in an animal biosafety level 3 laboratory. We present an alternative mouse model for acute lethal rickettsial disease, infecting intravenously C3H/HeN mice with *R. parkeri* Atlantic Rainforest strain (RpARF). We determined that infection with 1×10^6 and 1×10^7 viable RpARF organisms produced dose-dependent severity, whereas infection with 1×10^8 viable bacteria resulted in a lethal illness. The animals became moribund on day five or six post-infection. The lethal disease was characterized by ruffled fur, erythema, labored breathing, decreased activity, and hunched posture, which began on day three post-infection and coincided with the peak bacterial loads. Significant splenomegaly, neutrophilia, and thrombocytopenia were observed. Pathology was characterized by mural and valvular endocarditis, mononuclear cellular infiltration between the renal tubules and intertubular capillaries, meningitis, interstitial pneumonia, and cellular infiltration in liver. RpARF organisms were associated with vessels of the microcirculation in areas of pathological damage in the heart, kidney, brain and lung by immunohistochemical staining. In the liver, RpARF was observed in hepatocytes and mononuclear cells in addition to endothelial cells. The greatest advantage of this inbred mouse model is the ability to investigate immunity and pathogenesis of rickettsiosis with all the tools available at biosafety level 2.

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Contribution of Host Lipid Metabolism to Pathogenicity of *Rickettsia* During Infection in Mammalian Cells

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Vector Borne Disease Laboratories, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, USA

Abstract

Previous work in our lab has shown that pathogenic *Rickettsia* species are capable of replicating within a variety of mammalian target cells including endothelial cells, and monocytes and macrophages. Whereas much is known about *Rickettsia*-endothelial cell interactions, very little is known about the *Rickettsia*-macrophage relationship. A recent proteome analysis on macrophage-like THP-1 cells during infection by *R. conorii* indicated that several metabolic pathways in host cells are upregulated during infection. Among these proteins are those involved in lipid metabolism pathways such as fatty acid synthesis (FAS) and fatty acid oxidation (FAO). To investigate if host lipid metabolic pathways play a role in infection of host cells by pathogenic *Rickettsia* species, we utilized independent pharmacological inhibitors of fatty acid synthase (FASN) and FAO in macrophage-like THP-1 cells and determined the growth of *R. conorii* by quantitative PCR (qPCR) and fluorescent microscopy. In addition, we utilized FASN specific RNAi to ensure that any effects on rickettsial growth would be attributed to FASN function. These experiments were also performed in the human endothelial cell line, EA.hy926, to determine if pathogenic *Rickettsia* species require FASN activity in other mammalian cells. *R. conorii* growth is significantly inhibited in macrophage-like THP-1 cells when treated with specific pharmacological inhibitors of FASN and FAO, as well as with inhibition of FASN by RNAi. However, *R. conorii* growth is not disrupted in EA.hy926. These results demonstrate that the utilization of fatty acid metabolism pathways is required for effective *R. conorii* infection and replication of phagocytic cells *in vitro*.

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Role of rickettsial O-antigen polysaccharide in parasitic lifecycle, pathogenesis, and protective immunity

Hwan Keun Kim¹, Ranjan Premaratna², Dominique Missiakas¹, Olaf Schneewind¹

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Abstract

Rickettsia conorii is an obligate intracellular pathogen with a parasitic lifecycle involving both vertebrate and invertebrate hosts. Ticks serve as the natural vector and reservoir for *R. conorii* and transmit boutonneuse fever to humans during a bloodmeal. While spreading through the bloodstream, *R. conorii* primarily targets vascular endothelial cells and causes systemic vasculitis. For successful survival in both arthropod and mammalian hosts, *R. conorii* must induce unique sets of genes required for parasitic lifecycle, disease transmission, immune evasion, and virulence. However, little is known about these genetic determinants or the underlying molecular mechanisms. Here, we developed an *in vitro* transposon mutagenesis system for *R. conorii*, isolating chloramphenicol-resistant variants with random insertional lesions. Endowed with this technology, we demonstrate that mutations in the conserved *polysaccharide synthesis operon (psa)* abolish the biosynthesis of O-antigen polysaccharide that serves as an epitope for the serological diagnosis of rickettsioses (Weil–Felix serology). Our results show that the *psa* variants are defective in the assembly of outer membrane proteins, invasion of host cells, and boutonneuse fever pathogenesis. Unlike wild-type *R. conorii*, the *psa* variants fail to elicit bactericidal Weil–Felix antibodies that are likely to provide correlates for protective immunity against rickettsial diseases.

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Understanding the molecular determinants of autoprocessing of APRc, the retropepsin-type protease from Rickettsiae

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Abstract

Rickettsiae contain a highly conserved membrane-embedded retropepsin-type protease-encoding gene. We have previously shown that the encoded soluble domain of *R. conorii* protease homolog (APRc) shares several enzymatic properties with viral retropepsins and that its monomer follows the canonical fold observed in all retropepsins, either of viral or eukaryotic origin. Our data also suggest that APRc undergoes multistep processing to release its mature form, in which the protease precursor acts both as the enzyme and substrate at the same time. This is not unexpected in retropepsin-like proteases but, as for HIV-1 protease, the precursor processing mechanism(s) of these enzymes remain an intriguing puzzle where many fundamental questions are yet to be answered. Since APRc likely represents an ancestor of retropepsins, it emerges as a model system to further advance our current understanding of autoprocessing mechanisms in retropepsin-type proteases. In this work, we have expressed several truncated versions of APRc in *E. coli* to address different questions: i) How longer vs. shorter N-terminal regions impact the autoprocessing capacity of APRc? Is the autoprocessing activity differentially affected by non-native N- or C-terminal flanking sequences? What are the critical residues that abolish precursor autoprocessing activity? How different are the proteolytic properties of the precursor(s) vs. the mature form? Overall, our results support the concept that the APRc precursor and the mature protease are quite different in their catalytic properties, suggesting higher structural plasticity of the precursor and its contribution for autoprocessing-induced conformational changes that impact activation of the mature form of APRc.

ASR 2018 Business Report

The 29th meeting of the American Society for Rickettsiology was held at the Hyatt Regency in Milwaukee, Wisconsin. The hotel offered a great venue with views of downtown Milwaukee, and the city offered great food and plenty to see within walking distance. Conference attendees enjoyed good weather as they greeted colleagues and friends.

The Division of Continuing Education at Kansas State University, along with the Scientific Workshop and Organizing Committee, worked very hard to ensure a well-organized and stimulating meeting. The meeting was attended by 174 participants from 16 countries, and 25 travel grants were awarded. The conference began on June 16th with an opening reception that included remarks by ASR president Dr. J. Stephen Dumler. Dr. Arthur Allen delivered an insightful historical keynote address discussing the history of typhus, focusing on the critical contributions of Drs. Rudolf Weigl and Ludwik Fleck.

The remainder of the meeting was an excellent forum for discussion of the most recent developments in Rickettsial research. Concurrent scientific sessions were held to accommodate the number and variety of presentations. Poster sessions provided networking opportunities and showcased many up and coming Rickettsiologists. New officers were elected for 2018-2019, including Kelly Brayton as the new ASR President. Additionally, two long time leaders in our society, David Walker and David Wood, were appointed honorary members. The ASR thanks you both for your continual support. Congratulations on this well-deserved honor!!

Finance Report:

2017 Balance Forward: \$46,000

2018 Income: \$105,742.85

2018 Expenses: \$103,916.46

Remaining balance: \$47,826.39

The 30th Meeting of the American Society for Rickettsiology

2018-2019 Executive Committee

President

Kelly Brayton, PhD
Department of Veterinary Microbiology
and Pathology
Washington State University

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Department of Microbiology and
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Indiana University School of Medicine

Past President

J. Stephen Dumler, MD
Joint Department of Pathology
Uniformed Services University of Health Sciences
Walter Reed National Military Medical Center

2019 Organizing Committee

The organizing committee consists of the Executive Council members plus the following:

Maria Galletti	Centers for Disease Control and Prevention
Roman Ganta	Kansas State University
Ted Hackstadt	NIH-Rocky Mountain National Labs
Mike Minnick	Montana State University
Susan Noh	United States Department of Agriculture
Jeanne Salje	Rutgers University/Oxford
Jim Samuel	Texas A&M University
Dana Shaw	Washington State University

2019 Nominations Committee

Roman Ganta	Kansas State University
Uli Munderloh	University of Minnesota

2019 Scientific Committee

Sharanjeet Atwal*	Rutgers University/Oxford
Jason Carlyon	Virginia Commonwealth University
Katelynn Doiron*	University of Arkansas for Medical Sciences
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Ed Shaw	Oklahoma State University
Isaura Simoes	University of Coimbra
Muna Solymon*	Washington State University
Ying Wang*	Kansas State University

* A special thanks to our student/postdoctoral members

† Dr. Wei-Mei Ching was a member of the Scientific Committee until her passing

ASR Meeting Coordinator

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Travel Awardees

Gerardo Alvarez-Hernandez - University of Sonora

Esteban Arroyave - The University of Texas Medical Branch

Thomas Burke - University of California, Berkeley

Mebratu A. Bitew - The University of Melbourne

Ian Cadby -- University of Birmingham

Michael Dodd - Washington State University

Katelynn Doiron - University of Arkansas for Medical Sciences

Amanda Dragan - University of Arkansas for Medical Sciences

Liam Fitzsimmons - National Institute of Allergy and Infectious Disease

Ryan Green - Virginia Commonwealth University School of Medicine

Inaya Hayek - Mikrobiologisches Institut, Universitätsklinikum Erlangen

Weiyang Huang - The Ohio State University

Ilirjana Hyseni - Department of Pathology, University of Texas Medical Branch

Tatiana Mordente Clemente - Indiana University School of Medicine

Madison Rogan - University of Texas Medical Branch

Baleigh Schuler - Indiana University School of Medicine

Victoria I. Verhoeve - Department of Biology, West Virginia University

Shaun Wachter - The University of Montana

Jianyang Wang - Henry M. Jackson Foundation

Wenqing Zhang - The Ohio State University

In Memory of
Karin Aistleitner, PhD
1984-2019



It is with great sadness that I inform you of the passing of Dr. Karin Aistleitner on Sunday, March 31, following an extended illness. Karin was a great scientist and wonderful friend. Karin obtained her Ph.D. from the University of Vienna working with environmental chlamydia. She spent approximately two years doing work with the German Army. She joined the Host-Parasite Interaction Section at the Rocky Mountain Laboratories in January of 2017. Here she contributed immensely to work on the agent of Rocky Mountain spotted fever. Her enthusiasm, creativity, and hard work were an inspiration to all of us. RML has lost one of its best and brightest.

--Ted Hackstadt

In Memory of
Wei-Mei Ching, PhD
1949-2019



We regret to announce the untimely passing of Dr. Wei-Mei Ching at age 69. Dr. Ching was a Senior Scientist in the Viral and Rickettsial Diseases Department of the Naval Medical Research Center (NMRC) (formerly the Naval Medical Research Institute-NMRI-at Bethesda, Maryland), Silver Spring, Maryland since 1986 and also an Adjunct Professor in the Department of Preventive Medicine and Biostatistics at the Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland. Dr. Ching was an ASR member since 1989. Wei-Mei Ching received her B.S. in 1970 from the Department of Agriculture Chemistry, National Taiwan University and obtained her Ph.D. in Biochemistry from the University of Pennsylvania in Philadelphia in 1977. It is through her broad interest and contributions to the field that Wei-Mei Ching became friends to many of us. She was unselfish, kind and always ready to help nurture and cultivate young minds, and query older ones about their assumptions and priorities for the field. Her mind and personality was full of curiosity and unmatched enthusiasm. She was always encouraging, considerate, humble, and very determined. Dr. Ching directed her laboratory with kindness while also maintaining an uncompromising standard to ensure that the work was done in the best way possible. Her life was an exemplary one, full of peace, gratitude, faith, hope, and especially love of her family and colleagues.