“The Southern HIV & Alcohol Research Consortium (SHARC), led by Robert L. Cook, MD, MPH, has a mission to reduce HIV transmission and to improve health outcomes among persons affected by alcohol and HIV in Florida. The cover image shows clusters of persons with HIV infection in Florida, who have nearly identical viral genetic sequences. The work comes from a collaboration of SHARC with the Data Intelligence Systems Laboratory, led by Mattia Prosperi, MEng, PhD, and the Florida Department of Health.”

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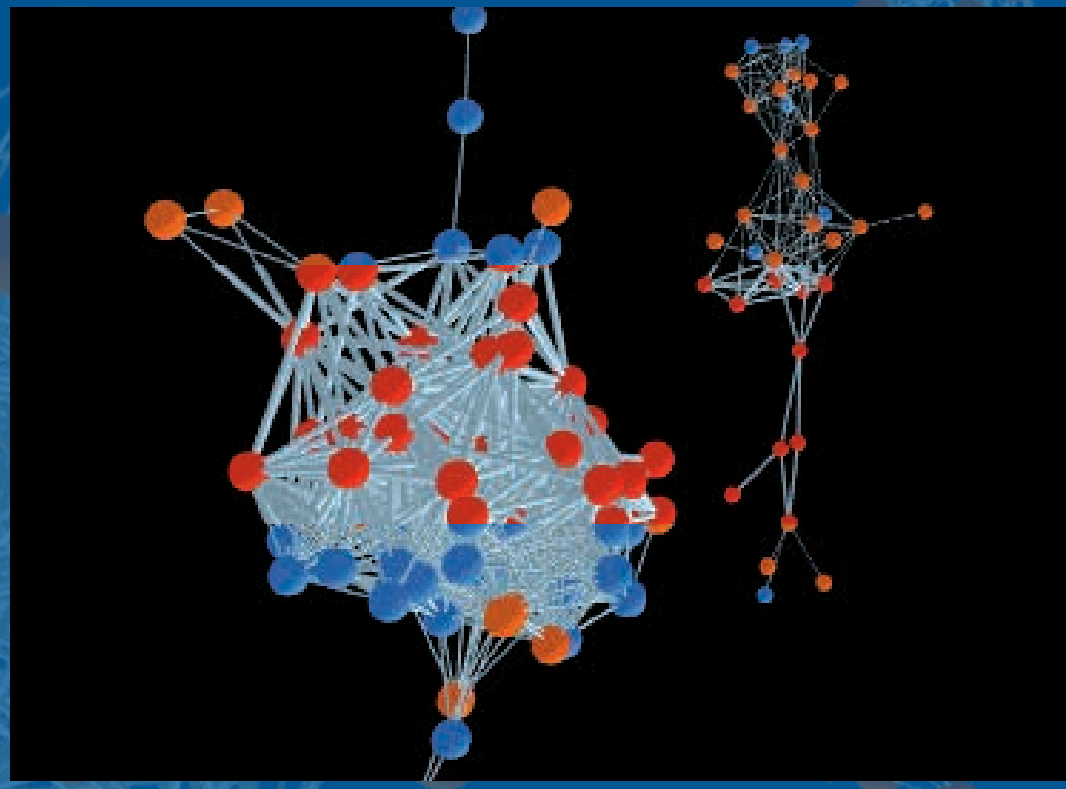
EPI RESEARCH DAY Book of Abstracts 2020

EMERGING PATHOGENS INSTITUTE

RESEARCH DAY



Book of Abstracts

FEBRUARY 2020

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Letter from the Director

Welcome to the thirteenth annual EPI Research Day! As you look through the abstracts in this book, and view the associated posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators at the University of Florida. In keeping with the interdisciplinary nature of EPI, authors come from seven different UF Colleges. We are also pleased to welcome a number of investigators from outside of UF, including authors/collaborators and guests from other Universities in Florida and neighboring states, and local, state, and federal agencies.

This year we have the honor of introducing you to two outstanding investigators who will be providing keynote talks during our afternoon session:

Dr. Kathryn Hanley is Professor of Biology at New Mexico State University. Her research is in the area of ecologic niche modeling, with a particular focus on arboviruses and their transmission, including sylvatic transmission cycles.

Dr. Philippe Sansonetti, from Institute Pasteur, was originally scheduled to be our second speaker. Unfortunately, due to a family emergency, he was not able to join us today. However, I am very pleased to say that **Dr. Derek Cummings**, from UF's Department of Biology, was kind enough to step in at the last minute to give the talk. Dr. Cummings is a UF Preeminence Professor and is internationally recognized for his work on transmission of infectious diseases, with a particular focus on dengue and other arboviruses. We are honored to have him and appreciate his willingness to speak on such short notice.

Please visit our website, www.epi.ufl.edu, to join our list-serves, and to keep up with our news, events and seminars throughout the year. And thanks for coming!

J. Glenn Morris, Jr. M.D., M.P.H. & T.M.
EPI Director and Professor of Medicine

Schedule of Events

9:00 AM – 10:00 AM	Registration, Breakfast, and Poster Setup *Reitz Union – Grand Ballroom*
10:00 AM – 1:00 PM	Poster Session *Presenters, please stand by your posters*
12:00 PM – 12:45 PM	Lunch *Reitz Union – Grand Ballroom*
12:45 PM – 1:00 PM	Keynote Assembly *Reitz Union – Grand Ballroom*
1:00 PM – 1:10 PM	Welcome *Dr. David Nelson, MD* *Senior Vice President* *for Health Affairs, UF* *President, UF Health* Introductions *Dr. J. Glenn Morris* *Director, EPI*
1:10 PM – 3:15 PM	Keynote Speeches
3:15 PM – 4:00 PM	Poster Removal

1:10 – 2:10

Kathryn A. Hanley, Ph.D

Regents' Professor
Department of Biology
New Mexico State University

"*Waiting in the Wings: Spillover, Spread and Spillback of Mosquito-Borne Viruses*"

2:10 – 3:10

Derek A.T. Cummings, Ph.D

Professor, Department of Biology, University of Florida
Adjunct Professor, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

"*The Ecology of Dengue: Changes from Intervention, Climate and Competition*"

01. A LIMITED CONSORTIUM OF MOUSE GUT FLORA CONFERS RESISTANCE AGAINST CLOSTRIDIOIDES DIFFICILE INFECTION

James Martin - Department of Medicine, College of Medicine, University of Florida; **Daniel Marquina** - College of Liberal Arts and Sciences, University of Florida; **Gurjit Sidhu** - Department of Medicine, College of Medicine, University of Florida; **Joan Whitlock** - Department of Medicine, College of Medicine, University of Florida; **Gary Wang** - Department of Medicine, College of Medicine, University of Florida

Introduction: Clostridioides difficile infection (CDI) is the most common cause of healthcare-associated infections in US hospitals. C. difficile is responsible for nearly half a million cases of diarrhea and colitis per year resulting in 30,000 deaths. While standard antibiotic therapy is effective, 15-30% of patients will develop a subsequent recurrence. Antibiotics and gut dysbiosis render patients susceptible to CDI, but restoring the gut microbiota by fecal microbiota transplant cures CDI. However, the specific gut microbes critical for C. difficile resistance are not known.

Methods: "Firmicutes" donor mice (FD) were generated in ex-GF mice whose gut microbiome was restricted to the class Clostridia. Fecal extracts obtained from FD mice were either diluted serially or passaged anaerobically on common microbiological media. Culturing attempts yielded 33 isolates, and 25 unique taxonomic units (OTUs) were confirmed according to 16S rRNA sequencing on the Illumina Mi-Seq platform. The microbiome of GF mice was then reconstituted and orally gavaged in pairs with serially diluted FD fecal in descending scale. For each FD dilution pair, one animal also received a supplement of the 33 isolates. After a period for stable colonization, they were challenged with a virulent strain of C. difficile VPI 10463.

Results: Following C. difficile challenge, GF mice engrafted with the FD 1:66 dilution that were also supplemented with the 33 isolates were completely resistant to CDI, negative for both toxin and pathogen colonization. The 1:66 dilution by itself and all decreasing dilutions and

their isolate pairs were susceptible to CDI and unable to prevent long-term C. difficile colonization and toxin expression.

Conclusion: Both the combined treatments were predominated by members of Ruminococcaceae and Lachnospiraceae, reinforcing our observation that members of these two families alone are competent to reproduce resistance. Where neither the 33 isolates, nor the 1:66 FD dilution by themselves were able to confer resistance against CDI, as a combination they conferred complete protection in a GF mouse. Future culturing efforts concentrated on the 1:66 FD subset could capture missing OTUs, which combined with the 25 OTUs already obtained, encapsulate the whole microbial element responsible for protection against CDI. A minimal consortium of isolates could then be dissected for genetic and metabolomic contributions that underlie microbial resistance to CDI.

02. CAMPYLOBACTER COLONIZATION, ENVIRONMENTAL ENTERIC DYSFUNCTION, STUNTING, AND ASSOCIATED RISK FACTORS AMONG YOUNG CHILDREN IN RURAL ETHIOPIA

Dehao Chen - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Sarah L. McKune** - Department of Environmental and Global Health, Center for African Studies, College of Public Health and Health Professions, University of Florida; **Nitya Singh** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Background: Undernutrition has been identified as an underlying cause in 45% of all under-five mortality. Livestock farming provides a possible mechanism by which smallholder farmers can meet their household need for animal source foods (ASF) which may reduce the risk of stunting. However, direct/indirect contacts with domestic animals may increase colonization by Campylobacter spp., which has been associated with Environmental Enteric Dysfunction (EED) and stunting.

Methods: A cross-sectional study involving 102 randomly selected children between 12 and 16 months of age was conducted in rural

eastern Ethiopia. Data on anthropometry, EED biomarkers, Campylobacter colonization, socio-demographic variables and hygiene was collected between September and December 2018.

Results: The prevalence of EED and stunting was 50% (40% - 60%) and 41% (32% - 51%), respectively. 56% of children had consumed some ASF in the last 24 hours. 47% and 50% of children had diarrhea and fever in the past 15 days. 55%, 63%, 71% or 43% of households owned at least one chicken, cattle, goat, or sheep; 54 (53%) households kept chickens indoor overnight and only half of these confined the animals. Sanitation was poor, with high levels of unimproved latrines and open defecation. Most households had access to an improved source of drinking water. The prevalence of Campylobacter colonization was 50% (41% - 60%) by PCR. In addition to the thermotolerant species C. jejuni, C. coli and C. upsaliensis, non-thermotolerant species related to C. hyointestinalis and C. fetus were frequently detected by Meta-total RNA sequencing (MeTRS).

Conclusions: There was a high burden of Campylobacter in the study area, which may be associated with the high prevalence of EED and stunting though the associations were not significant in this small sample. Further studies are necessary to better understand reservoirs and transmission pathways of Campylobacter spp. and their potential impact on child health.

03. CHANGE IN IGA FOLLOWING ROTAVIRUS VACCINATION IS NOT CORRELATED WITH PROTECTION AGAINST SYMPTOMATIC ROTAVIRUS DISEASE: SECONDARY ANALYSIS OF A ROTAVIRUS VACCINE TRIAL IN CHILDREN IN NIGER

Matt Hitchings - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Sheila Isanaka** - Harvard T.H. Chan School of Public Health; **Rebecca Grais** - MSF Epicentre; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

There is a consistently observed trend of lower vaccine efficacy against severe rotavirus gastroenteritis measured by rotavirus (RV) vaccine trials in lower-income settings, compared to those in higher-income settings. Maternal antibodies (both transplacental and from breastfeeding) may provide protection against RV infection, but might interfere with immunogenicity of the vaccine and therefore with vaccine efficacy. This interaction is offered as one explanation for lower vaccine efficacy in lower-income countries. Immunogenic response to vaccination is measured by change in serum IgA titre, a correlate of protection for RV infection. Commonly, seroconversion is defined as >3-fold rise in IgA titre from pre- to post-vaccination, although some studies define seroconversion as change from IgA<20 to IgA≥20 pre- to post-vaccination. Such binary definitions do not fully capture inherent variability in antibody titres and assay variability that may be captured when exploring the continuous titre. We apply a mixture model to the change in IgA titre among a sub-cohort of 1,545 children enrolled in an RV vaccine trial conducted in Niger. Assays were performed before and 28 days after the completed vaccination course. We use the mixture model to identify pre-vaccination variables associated with impaired IgA response. Finally, we perform a mediation analysis to assess the proportion of vaccine efficacy that is mediated by changes in IgA after vaccination. Our results appear contradictory: pre-vaccination serum IgA in children, along with IgG and mother's breast milk IgA, is negatively associated with rise in IgA after vaccination and with vaccine efficacy. This finding suggests a correlation between serum IgA activity and vaccine protection. However, the mediation analysis demonstrates that only 2% of the vaccine effect is mediated through post-vaccination IgA titre. A single measurement of

serum IgA taken 28 days post-vaccination is not sufficient to capture dynamic antibody changes that are correlated with protection in this population.

04. CHITOSAN MICROPARTICLES SHOW PROMISING POTENTIAL AS A CHEMICAL TREATMENT AGAINST BACTERIOPHAGE MS2, A HUMAN NOROVIRUS SURROGATE

Candace Barnes - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Rebecca Barber** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Robyn Louis** - Department of Psychology, College of Liberal Arts and Sciences, University of Florida; **Anita Wright** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Melissa Jones** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Naim Montazeri** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Human norovirus is the leading cause of acute viral gastroenteritis worldwide and a public health concern because of its low infectious dose and persistence in the environment. Water plays a major role in the transmission of norovirus to humans, and effective decontamination of water can control the spread of the pathogen. Chitosan is a polysaccharide derivative of chitin and has shown promising antibacterial activity; however, limited research has been performed on its application for the antiviral treatment of water. In this study, we investigated the antiviral activity of chitosan microparticles (CM) in suspension against bacteriophage MS2, a cultivable surrogate for human norovirus. CM was generated through the ionic gelation of chitosan solution using sodium sulfate as a cross-linker while sonicating, quality assessed against the MS2 host E. coli strain using macrodilution broth method and size-characterized by dynamic light scattering. The CM preparation had 6% (w/v) dry weight and a particle size range of approximately 0.5-1.0 µm. Quality assessment of CM revealed a minimum inhibitory concentration

of 0.006% (w/v) with no interference in infectivity assays. The impacts of CM against virus infectivity and genome integrity were assessed with plaque assay and reverse-transcriptase quantitative PCR (RT-qPCR), respectively. The infectious titer of MS2 with 0.3% CM immediately decreased to the limit of detection of 1.85 log10 PFU/ml, and viral genome with CM concentrations up to 0.01% decreased to the limit of detection of 1.12 log10 RT-qPCR units at 0-hour contact time. Further research focuses on continuing mechanistic studies using a plate-based thermal release assay (PaSTRy) to assess virus capsid and RNA stability in binding to CM, visualizing CM using electron microscopy, investigating the impact of CM on human norovirus, and assessing downstream applications in agricultural water.

05. COMPLETE NUCLEOTIDE SEQUENCE OF A TRANSMISSIBLE PLASMID ENCODING CTX-M1 TYPE CEFOTAXIMASES IN A S. HEIDELBERG STRAIN ISOLATED FROM A TURKEY

Thomas Denagamage - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Ananta Arukha** - College of Veterinary Medicine, University of Florida; **Subhashinie Kariyawasam** - College of Veterinary Medicine, University of Florida

Salmonella enterica serovar Heidelberg is among the top five serotypes associated with human salmonellosis. S. Heidelberg infections are more invasive than other nontyphoidal Salmonella infections requiring antibiotic treatment. Ceftriaxone, a third-generation cephalosporin is the drug of choice to treat invasive Salmonella infections in children and in adults with fluoroquinolone contraindications. Therefore, resistance to extended spectrum cephalosporins (ESCs) among nontyphoidal salmonellae is a major public health concern. Food-producing animals, in particular poultry, are considered the primary reservoir of ESC-resistant Salmonella. This implies a possible foodborne and zoonotic transfer of ESC-resistant Salmonella and their resistance genes to humans via the food chain, contaminated production environment, or animal handling. We have previously reported ESC- resistant S. Heidelberg strains, which harbor IncN/ST1 type plasmids that encode CTX-M1 type extended-spectrum beta lactamases (ESBL) or cefotaximases, from poultry. Here, we describe the complete nucleotide sequence of one of these CTX-M1 type cefotaximase-encoding plasmids, named pS_9079. The pS_9079 was

present in a S. Heidelberg strain isolated from the ceca of a turkey. The sequenced plasmid was ~42,400 bp in size and demonstrated ≥90% similarity to many other CTX-M1 plasmids identified in bacteria belonging to the family Enterobacteriaceae. The 875-bp blaCTX-M-1 gene that confers ESC resistance was located immediately downstream to an IS26 family transposase gene. Furthermore, conjugation experiments showed that the plasmid was capable of conjugative transfer. The IncN/ST1 plasmids have been responsible for extensive spread of blaCTX-M-1 in livestock, poultry, and humans in Europe. To our knowledge, this is the first report of the complete nucleotide sequence of a CTX-M1 type ESBL plasmid in a S. Heidelberg isolated from poultry in the United States. This study highlights the importance of One Health approach to combat antibiotic resistance, including ESC resistance, in bacteria.

06. DECLINE OF CHOLERA CASES CORRELATES WITH INCREASING ISOLATION OF NON-TOXIGENIC VIBRIO CHOLERAE O1 STRAINS FROM ENVIRONMENTAL RESERVOIR IN HAITI

Meer Taifur Alam - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Mohammad Shahnoor Hossain** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Sk Nazmus Sakib** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Vanessa Rouzier** - Les Centres GHESKIO, Port-au-Prince, Haiti; **Mohammed Rashid** - Emerging Pathogens Institute, University of Florida; **Valery Madsen beau de Rochars** - Department of Health Services Research, Management and Policy, Emerging Pathogens Institute, University of Florida; **Jean William Pape** - Les Centres GHESKIO, Port-au-Prince, Haiti; **J. Glenn Morris Jr.** - Department of Medicine, Emerging Pathogens Institute, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Environmental Management Systems Institute, University of Florida

Cholera, a profuse diarrheal disease, caused by toxigenic strains of Vibrio cholerae O1 was absent in Haiti for 100 years. However, on October 2010 the disease was accidentally introduced by a Nepalese peace keeping troop potentially carrying asymptomatically toxigenic V. cholerae O1

Ogawa serotype strain in Haiti. Although cholera is a water-borne disease, some investigators previously reported that there was no environmental or water contamination of toxigenic V. cholerae O1 in Haiti. In contrast, beginning 2012 we have monitored a series of sentinel environmental sites in Haiti; based on the surveillance we earlier reported that toxigenic V. cholerae O1 strains have persisted and established environmental reservoirs in Haiti with rainfall and temperature contributed to the bloom of the pathogen in aquatic reservoirs. For unknown reasons, cases of cholera have dramatically declined in Haiti between 2017 and 2019 (estimated 17, 899 total suspected cases with 203 deaths was reported by MSPP and PAHO) compared to preceding two years, including 2015 and 2016 (estimated 77,466 suspected cases with 769 deaths). Of particular note, we observed that majority of V. cholerae O1 Ogawa serotyped switched to Inaba serotype at the end of 2015. Interestingly, we also observed a significant increase of isolation of non-toxigenic V. cholerae O1 strain from aquatic reservoirs between 2017 and 2019 compared to 2012 to 2016. We isolated 14 (82.4%) of 17 V. cholerae O1 strains as non-toxigenic O1 strains between 2017 and 2019 compared to only 3 (9.4 %) of 32 V. cholerae O1 strains as non-toxigenic strains between 2012 and 2016. Whole genome sequencing and preliminary bioinformatics analysis on the subset of non-toxigenic O1 strains revealed that all of the strains either completely or almost completely lost all of the known V. cholerae virulence islands, including Tcp, CTX and VSP islands. Moreover a few of them also lost wbeT gene from O-antigen biosynthetic region rendering these strains as only polyvalent seropositive V. cholerae O1 strains. We are currently investigating if the loss of virulence islands correlates to simple retrieval of the islands from V. cholerae's genome or horizontal gene transfer (HZT). Our results underscore the importance of continuous environmental monitoring of toxigenic V. cholerae in environmental setting with follow-up WGS and bioinformatic analysis to track the pathogen movement and evolution and thereby monitoring cholera epidemics in cholera endemic countries as in as in Haiti.

07. DYNAMICS OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI IN BEEF CATTLE THROUGHOUT THE LIFESPAN

Lin Teng - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Peixin Fan** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Madeline R. Steck** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Christina Boucher** - College of Engineering, University of Florida; **J. Glenn Morris Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

The emergence of Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli has become a great concern to public health because they cause treatment failure of third-generation cephalosporins, which are widely used in human hospitals to treat bacterial infections. Recently, it has been shown that cattle raised without antibiotics carry ESBL-producing E. coli in the gut. However, colonization dynamics and characteristics of these pathogens in cattle are not known. In this study, we investigated colonization dynamics throughout the lifespan of beef cattle by measuring the prevalence of ESBL-producing bacteria in fecal samples collected from the recto anal junction of cattle (n=322) every 3 months from the birth to the slaughterhouse. As the cattle grew up on pasture, the prevalence of ESBL-producing E. coli increased from 10.9% in 0 month (M) to 20.4% in 3 M, and then decreased to 1.1% in 12 M. The average concentration of ESBL-producing E. coli decreased from 707 CFU/swab in 0 M to under detectable level in 12 M. A sub-portion of the cattle (n=108) were transported to a feedlot after 12 M of birth. In the feedlot the prevalence of ESBL-producing E. coli reached 100% (15 M and 18 M), and its average concentration rose to 240 CFU/swab (15M) and 249 CFU/swab (18M). Phylogenetic analysis of genomic DNA revealed that ESBL-producing E. coli in the feedlot showed more diverse sequencing types (STs) than those on pasture. Clonal variants were isolated from cattle on pasture (12 M) and feedlot (18 M), indicating the

transmission of ESBL-producing E. coli from pasture to feedlot occurs. Taken together, we report colonization dynamics of ESBL-producing E. coli throughout the lifespan of beef cattle that are affected by bacterial, animal, and environmental factors, shedding light to develop mitigating strategies for antibiotic resistance.

08. EXPLORING SPATIAL AND TEMPORAL EPIDEMIOLOGY OF SALMONELLOSIS IN FLORIDA

Xiaolong Li - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Nitya Singh** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Elizabeth M Beshears** - Florida Department of Health, Food and Waterborne Disease Program, Tallahassee, FL; **Jason L. Blanton** - Florida Bureau of Public Health Laboratories, Jacksonville, FL; **Jamie DeMent** - Florida Department of Health, Food and Waterborne Disease Program, Tallahassee, FL; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

Non-typhoidal Salmonella enterica infections cause a high disease burden in the United States with an estimated 1.35 million illnesses annually. In 2017, Florida reported 6,557 cases of salmonellosis, or an incidence rate of 31.9 per 100,000 which is considerably higher than the national average of 16.7 per 100,000. In this study, we examined the spatial and temporal patterns of surveillance data for a total of 61,518 salmonellosis cases reported between 2009 and 2018, downloaded from Florida Health charts (http://www.flhealthcharts.com/). In addition, metadata obtained from Florida Department of Health, Food and Waterborne Disease Program and Whole Genome Sequencing data obtained from Florida Bureau of Public Health Laboratories of 2,507 Salmonella isolates from 2017-2018 were used for descriptive epidemiology and serotype prediction. From reported data between 2009-2018 an annual incidence rate ranging from 27.8 to 36.0 per 100,000 population was observed with the highest incidence rate for children contributing 41.8% of total reported cases and a seasonal pattern was observed with the incidence peaking in September and October. The median polish method was utilized to investigate the variance of incidence rates between six regions in Florida across these ten years, suggesting that the Northeast and Northwest region had higher reported incidence rates, while reported

rates in the Southeast and South were gradually increasing over time. Salmonella Enteritidis (7.0%), Newport (6.4%), Sandiego (4.9%), Javiana (4.7%) and Typhimurium (4.2%) were the most prevalent serotypes in 2507 isolates sequenced during 2017-2018. Demographics of patients calculated from this dataset, suggested approximately similar female (52.5%) to male (47.5%) ratio and highest percentage of samples from children under 5 years of age (41.4%). Stool (84.7%) was observed as the major source of samples from which these strains were isolated. The number of sequenced cases accounted for 20.6% and 16.0% of total reported cases in 2017 and 2018, respectively. In conclusion, children under 5 years are the main affected population group by salmonellosis in Florida. The spatial and temporal patterns suggest that prevention strategies should be more focused on Southeast and South Florida where the incidence rates continue to rise.

09. GENOMIC EPIDEMIOLOGY OF TOXIGENIC VIBRIO CHOLERAE IN HAITI: A SWITCH IN SEROTYPE

Taylor Paisie - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Melanie Cash** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Meer Taifur Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **J. Glenn Morris Jr. -** Emerging Pathogens Institute, University of Florida; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

In October 2010, cholera appeared in Haiti for the first time in over 150 years. After the initial epidemic waves, cholera may now be endemic in Haiti, showing outbreak patterns associated with the rainy season. Since the start of the cholera outbreaks the Ogawa serotype dominated the cases of cholera in Haiti. Then in 2015, Inaba became the dominant serotype in Haiti, surpassing the number of cases caused from original

Ogawa serotype. The switch from Ogawa to Inaba is a common phenomenon in the V. cholerae genome, but typically if an Ogawa strain is circulating in the population and another outbreak of Inaba occurs, this is usually indicative of a separate introduction of the different serotype into the population. This single-source introduction of V. cholerae presents a unique opportunity to study the evolution and selective pressures acting on this microorganism. Performing phylodynamic analysis with genome-wide single nucleotide polymorphisms (SNP) allows us to investigate the underlying evolutionary processes at a remarkable resolution of the cholera epidemic in Haiti. This allows us to assess the evolutionary dynamics that are occurring in the V. cholerae genome that potentially generated this switch in serotype. The main driver causing the switch from Ogawa to Inaba are SNPs located in the wbeT gene. Our results propose that the V. cholerae strains circulating in Haiti have evolved from the introduction of their clonal, single-source of the Ogawa serotype to the new, unintroduced Inaba serotype. Outbreaks of different serotypes are indicative of a separate introduction, but our results promote that the Inaba serotype circulating since 2015 is the outcome of the initial Ogawa serotype evolving into the Inaba serotype. This could suggest that Haiti is becoming a source for toxigenic V. cholerae in the Caribbean and may lead to cholera outbreaks throughout the region.

10. HIGH PREVALENCE AND GENOMIC CHARACTERISTICS OF MULTI-DRUG RESISTANT EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI IN FERAL SWINE

Ting Liu - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Raoul K. Boughton** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Due to the potential role of wildlife as reservoirs and sources of antibiotic resistant pathogens, an increasing number of studies are focusing on antimicrobial resistance in wildlife. However, the genomic characteristics of antimicrobial resistance bacteria (ARB) in wildlife is largely unknown. To better understand the characteristics of ARB in wildlife, we isolated cefotaxime (third-generation cephalosporin antibiotic) resistant bacteria (CRB) from 224 fecal samples of feral swine. The prevalence of CRB was 37.5%. Ninety isolates were either CTX-M or CMY-2 positive by using a polymerase chain reaction method and they were selected for whole genome sequencing to characterize extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing E. coli in feral swine. Phylogenetic analysis revealed that these strains clustered into 15 groups that coincided with their sequencing types (STs). Twenty-four representative strains were selected based on their clusters in the phylogenetic tree and whole genome architecture for further comparative genomics and antimicrobial susceptibility test. All the representative isolates were multi-drug resistant and carried a variety of virulence genes, suggesting that these strains may threaten public health if they are transmitted to humans. Out of 24 strains, sixteen isolates contained the conjugative IncR plasmid in common. In addition, the same insertion element, IS5 and IS1380, were found near CTX-M-1 and CMY-2 genes respectively, which indicated that the plasmids were transmitted among these multi-drug resistant E. coli isolates. Our results provide critical knowledge to better understand the prevalence and genomic characteristics of these multi-drug resistant E. coli in feral swine that may serve as reservoir.

11. OCCURRENCE AND TRANSMISSION MECHANISMS OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lin Teng** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Kartikeya Cherabuddi** - Department of Infectious Disease, Emerging Pathogens Institute, College of Medicine, University of Florida; **Nicole Iovine** - Department of Infectious Disease, College of Medicine, University of Florida; **J. Glenn Morris Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Carbapenem is a class of highly effective antibiotic agent that is widely used for bacterial infection treatment. Enzymes produced by carbapenem-resistant Enterobacteriaceae (CRE) such as Klebsiella pneumoniae carbapenemase (KPC) and New Delhi metallo-beta-lactamase (NDM) can cause resistance to carbapenems. Hospital outbreaks of CRE have caused global threats to public health. Therefore, investigating the occurrence and possible transmission mechanisms of CRE among the patients in hospitals are of great importance. We isolated 24 CRE, including K. pneumoniae (12), Enterobacter hormaechei (7), Serratia marcescens (3), and Escherichia coli (2), from patients in UF Health Shands Hospital over a 19-month period (November 2017 to July 2019). Multiple-drug resistance (> 5 classes of antibiotics) of these strains, detected by antimicrobial susceptibility testing, offers the bacteria selective advantage due to antimicrobial use in hospitalized patients and thereby the hospital environment. Illumina whole-genome sequencing was performed to get insights of the genomic information of these strains. NDM was found in 3 E. hormaechei, 2 E. coli isolates and 5 K. pneumonia isolates, and KPC was in 5 E. hormaechei ,7 K. pneumonia and all 3 S. marcescens isolates. Maximum-likelihood phylogenetic trees constructed by core-genome SNPs suggested that clonal variants might exist among K. pneumonia, E. hormaechei and E. coli isolates. To

understand the loci of the NDM and KPC genes, PacBio sequencing was conducted on 12 selected isolates. Among those isolates, E. hormaeche KCJ3K13 and KCJ3K19, with genome size 5,147,607 and 4,936,130 bp respectively, carried the same plasmid (pKC45K) but interestingly encoded different NDM genes (NDM-1 and NDM-5). The comparison of genetic environments surrounding these 2 genes showed that the NDM-1 and NDM-5 genes were transpositioned into pKC45K at the same location by different insertion sequences, IS30 and IS5, respectively. Furthermore, we identified a plasmid carrying multiple resistance genes in E. hormaeche KCJ3K19. These results imply that IS element is a key factor spreading the NDM gene among Enterobacteriaceae; therefore, suppressing IS transposition could potentially reduce CRE outbreaks in the hospital setting.

12. QUANTITATIVE AND SPATIAL NETWORK ANALYSIS OF FOODBORNE ILLNESS COMPLAINT DATASETS IN FLORIDA, 2017-2018

Xiaolong Li - Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Amanda Sapp** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Nitya Singh** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Laura Matthias** - Florida Department of Health, Food and Waterborne Disease Program, Tallahassee, FL; **Chad Bailey** - Florida Department of Health, Food and Waterborne Disease Program, Tallahassee, FL; **Jamie DeMent -** Florida Department of Health, Food and Waterborne Disease Program, Tallahassee, FL; **Arie Havelaar** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida

According to CDC, approximately 10 million people suffer from foodborne illness caused by 31 major pathogens annually in the United States. However, traditional pathogen-specific surveillance systems can only capture a fraction of outbreaks or illnesses caused by foodborne pathogens. Consumer complaint systems can serve as an important supplement that can deal with non-reportable illnesses and identify foodborne disease outbreaks. A quantitative spatial network analysis was performed to understand the illness demographics and identify the hotspots of possible infections based on consumer complaints. Deidentified data from the Florida Complaint and Outbreak Reporting

System (FL-CORS) database for 2017 and 2018 were provided by Florida Department of Health, county level population data for the state of Florida were obtained from Floridahealthcharts.com and shapefiles for interactive mapping of Florida were obtained from myflorida.com. Data cleaning, manipulation and tabularization were performed using R 3.6. The spatial networks were constructed in Gephi software, and network metrics including degree and betweenness centrality were used to characterize the networks. Finally, the straight-line distances between residential and exposure area were calculated, and the association of distance with frequency of complaints was examined by a Poisson regression model. In total 5,504 complaints were filed in FL-CORS during 2017-2018. The overall incidence rate of complaints was 110 per million person-years for 2017 and 140 per million person-years for 2018. Involved counties, including both county of residence and county of possible exposure, were mainly observed in central and south Florida. Of all complaints requested in FL-CORS, 28.4% and 26.7% were confirmed as outbreaks in 2017 and 2018, respectively. However, of all reported outbreaks, 72.8% and 56.5% outbreaks in 2017 and 2018, respectively, were successfully confirmed according to the FL-CORS database. For most complaints, the county of presumed exposure was found same as the county of residence of the complainant, ranging between 42 and 100% of all complaints from a county. Network analysis aided to understand the quantitative and spatial patterns of exposure for residents from other counties. Orange county received the highest number of complaints from residents from other counties in both years, along with several areas in the Panhandle and north Florida. A significant negative association ($p = 0.001$) was found between frequency of complaints and travel distance. Consumer complaints are an important supplementary source to laboratory surveillance for identifying – mainly localized – foodborne disease outbreaks.

13. THE TRANSMISSIBLE GENE QNRVC PLAYS AN IMPORTANT AND UNDERSTATED ROLE IN FLUOROQUINOLONE RESISTANCE IN VIBRIO CHOLERAE

Ashton C. Marrazzo - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Masih Mahmoudisaber** - University of Montreal; **J.B. Harris** - Department of Pediatrics, Harvard Medical School; **R.C. LaRocque** - Department of Medicine, Harvard Medical School; **F. Qadri** - International Centre for Diarrhoeal Disease Research; **A. Camilli -** Tufts University; **S.B. Calderwood** - Massachusetts General Hospital; **B. Jesse Shapiro** - University of Montreal; **E.J. Nelson** - Department of Pediatrics, Emerging Pathogens Institute, College of Medicine, University of Florida

The fluoroquinolone (FQ) antibiotic ciprofloxacin is recommended for the treatment of Vibrio cholerae infection in severely dehydrated patients. FQ resistance has historically been attributed to topoisomerase (e.g. gyrA, parC) mutations, however the contribution of a FQ resistance gene, qnrV.cholerae, remains unclear. Determining the role of qnrVc is important because it is located on an integrating and conjugative element (ICE) and is found broadly across Gram negative taxa. We investigated the genotypic and phenotypic relationship between qnrVc and CIP resistance in V. cholerae clinical isolates. Isolates were collected from patients (N=67) during a single cholera outbreak in Dhaka, Bangladesh. Genomes were sequenced and resistance genes were identified using established databases (CARD, ResFinder). The minimum inhibitory concentration (MIC) for CIP was determined by logistic fit of growth (8 μg/ml – 0.00195 μg/ml) and strains were categorized as resistant (MIC ≥ 2 μg/ml) or sensitive (MIC ≤ 1 μg/ml). CIP resistance was found in 54% (36/67). All resistant isolates (36/36) contained qnrVc, which strongly correlated with resistance (McNemar's Chi-squared Test, p = <0.001, kappa = 0.5). The gene qnrVc was the only known FQ resistance determinant in eight isolates with an MIC range of 0.5-2 μg/ml, which is 1-4 fold above what was previously reported. These data suggest that phenotypic CIP resistance can occur via a mechanism independent from topoisomerase point mutations. We advocate that qnrVc be included and prioritized in FQ resistance surveillance.

14. YERSINIA INHIBITS INFLAMMASOME ACTIVATION AND PGE2 BIOSYNTHESIS FROM THP-1 MACROPHAGES IN A T3SS- YOPJ DEPENDENT MANNER

Austin Sheppe - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Mariola Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Prostaglandin E2 (PGE2) is an essential immunomodulatory lipid that is released by macrophages during Gram-negative bacterial infection. PGE2 has long been reported to have conflicting roles in immunity, and the PGE2's biological function in bacterial clearance is poorly understood. Recent research has focused on how PGE2 affects macrophage inflammatory IL-1β cytokine production by macro completes called inflammasomes. Inflammasomes drive activation and secretion of inflammatory cytokines such as IL-1β which results in a type of inflammatory cell death termed pyroptosis. Recently our lab discovered that Yersinia enterocolitica, a Gram-negative enteric pathogen, inhibits PGE2 biosynthesis and inflammasome activation in THP-1 macrophages in a pYV virulence plasmid-dependent manner. Exogenous PGE2 added to macrophages also limited loads of intracellular Yersinia enterocolitica 48 hours post-infection. Mutants of Yersinia enterocolitica unable to secrete virulence proteins via the use of the pYV-encoded type three secretion system (T3SS) were unable to inhibit PGE2 biosynthesis and secretion from THP-1 macrophages. To further understand the mechanism behind how Yersinia alters PGE2 secretion from macrophages, we investigated whether the sister pathogen Yersinia pseudotuberculosis also alters PGE2 secretion in a type three secretion system-dependent manner. We discovered Yersinia pseudotuberculosis also inhibits PGE2 biosynthesis and secretion from THP-1 macrophages albeit in a smaller magnitude compared to Yersinia enterocolitica. PGE2 secretion from macrophages treated with heat-killed or T3SS deficient Yersinia pseudotuberculosis was inhibited by the addition of a MAPK inhibitor but not a p38- or JNK-selective inhibitors. Further investigation revealed a YopJ catalytically inactive mutant of Yersinia pseudotuberculosis induced similar levels of PGE2 secretion compared to heat-killed or T3SS deficient bacteria. YopJ has been shown to inhibit MAPK signal transduction in macrophages by

transacetylation of residues that prevent phosphorylation and activation of downstream kinases. In summary, we propose that Yersinia uses YopJ to inhibit MAPK signal transduction as a potential mechanism to inhibit PGE2 biosynthesis and secretion from macrophages during Yersinia infection.

15. A PILOT STUDY TO ESTIMATE INFLUENZA AND OTHER RESPIRATORY PATHOGEN TRANSMISSION IN A SENIOR CITIZEN COMMUNITY

Brooke Borgert - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Matt Hitchings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Kyra Grantz** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **JD Ball** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **AY Shir** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **YP Vaughan** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

People 65 years and older experience severe clinical manifestations of influenza. For this reason, the Centers for Disease Control and Prevention prioritizes this population to receive annual influenza vaccinations. However, a widely understudied dynamic contributing to disease introduction and potential control methods are the unique social mixing patterns of senior citizens in assisted living facilities. This study aimed to survey a retirement community in Gainesville, FL to assess feasibility of conducting an influenza surveillance study in a retirement community setting, determine the viral and immunological makeup of the population during the 2017-2018 influenza season, and investigate the social risk factors for acquiring influenza or influenza like illness (ILI). To achieve this, nasal swabs and surveys were collected throughout the study from 47 staff members and 191 residents. A baseline and end of study nasal swab was collected to assess changes in the immune response of participants. Swabs were also collected weekly from participants who reported ILI's and participants chosen as controls. We tested samples using a multiplex PCR that detected multiple respiratory viruses including, coronavirus, influenza A-H3N2, respiratory syncytial virus (RSV), human rhinovirus/

respiratory enterovirus, human metapneumovirus, parainfluenza, and influenza B. A generalized linear model was used to identify risk factors associated with PCR confirmed viral infection. IgA responses to twelve influenza viruses (4 H1N1, 4 H3N2, 1 H7N7, and 3 influenza B viruses) from pre to post-season swabs were measured using ELISA. Significantly higher increases in IgA were measured for subtypes that people were infected with compared to subtypes that people were not infected with. Additionally, across all subtypes, individuals who had a positive infection experienced significantly higher increases in IgA compared to people not infected. Overall, the results of this study indicate the potential of IgA as a correlate of infection and demonstrated that a larger study in this population is not only feasible but necessary to continue to learn about influenza and ILI's for at-risk populations in order to inform policy and control mechanisms of transmission.

16. ILINET PROGRAM REVIEW FROM A COUNTY HEALTH DEPARTMENT-LEVEL PERSPECTIVE

Emily Dougherty - Florida Department of Health; **Julia Monroe** - Florida Department of Health; **Karen Elliott** - Florida Department of Health

Background: Influenza (flu) is a contagious respiratory illness caused by influenza viruses. Influenza-like illness (ILI) is defined as a fever greater than or equal to 100 degrees Fahrenheit and sore throat and/or cough in the absence of another known cause. Influenza viruses are continuously changing, which in turn changes the yearly influenza vaccine components. To keep up with the ever-changing virus and vaccine, the Centers for Disease Control and Prevention (CDC) has partnered with state and county health departments (CHDs) on a surveillance program called ILINet. In Florida, the CHDs coordinate the recruitment and retention of local outpatient health care providers for the program. Enrolled providers submit weekly numbers of total patients seen and patients with ILI by age group to the ILINet website. They also are asked to send five specimens from patients with ILI to the state laboratories each week. The state public health laboratories do initial testing and then send positive specimens to the CDC. The CDC recommends states recruit one participating primary care provider per 250,000 population. There are more than 2,600 providers across the 50 states, including Puerto Rico, District of Columbia and the U.S. Virgin Islands, who report to the ILINet.

Based on Florida's overall population, there should be around 85 participating providers. Florida currently has 117 providers, but only 66 are actively reporting and not all counties are represented equally. The information collected by this program contributes to the understanding of current influenza activity levels, determining what virus strains are currently circulating, detecting antiviral resistance, and selecting influenza virus strains to be included in the annual vaccine.

Methods: Qualitative data were collected by a survey that was sent to CHD flu coordinators in November 2019. The goal of the survey was to let coordinators have an opportunity to express challenges they face, share successful provider recruitment techniques they have come across, and brainstorm ideas on how to improve this program. An additional survey question was asked to gather feedback from providers about the program.

Results: Out of 67 counties, 25 were represented by 18 flu coordinators in the survey results. Sixty-seven percent of participating flu coordinators expressed facing challenges while implementing the program in their county or counties. Most of those flu coordinators shared similar challenges, which include providers being too busy to implement the program at their facility, CHD staff not having time to dedicate to recruitment, and providers who are interested in participating but unable to decide without consulting with administration because they are owned by a larger health system. Only three flu coordinators had successful recruitment techniques to share and four had ideas on how to overcome these challenges. Successful recruitment techniques and ideas on how to overcome challenges included working with the health care system's administration to recruit new facilities, face-to-face meetings with providers, holding flu information sessions at provider's offices, and sending weekly flu surveillance reports to providers.

Conclusion: The ILINet program can be a useful surveillance method for understanding the current flu activity, detecting antiviral resistance, and contributing to the selection of virus strains for next season's vaccine. However, to provide the desired situational awareness statewide, recruitment and retention of ILINet providers needs to be improved. New more effective strategies for influenza specimen acquisition and situational awareness of influenza activity also need to be explored.

17. MAPPING THE GLOBAL DISTRIBUTION OF MIDDLE EAST RESPIRATORY SYNDROME BASED ON MACHINE LEARNING MODELS, 2012–2018

Anran Zhang - Department of Epidemiology, Shandong University; **Yang Yang** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Liqun Fang** - The State Key Laboratory of Pathogen and Biosecurity , Beijing Institute of Microbiology and Epidemiology

Background: The ongoing circulation of the Middle East respiratory syndrome (MERS), an emerging zoonotic infectious disease with a relatively high case fatality rate, is a continuing threat to global public health. However, determinants for its pathogenicity, spread and ecology remain poorly understood.

Methods: We collected surveillance data on confirmed MERS cases during September 2012 to October 2018 worldwide mainly from the World Health Organization and Ministry of Health, Kingdom of Saudi Arabia, and geocoded the cases up to the second-level administrative divisions. Socioeconomic and environ mental drivers for the diffusion of MERS were identified using a survival analysis. Ecological factors for the persistence of MERS were assessed using machine learning models.

Results: A total of 2,139 confirmed MERS cases were reported worldwide, with 90% in the Middle East. Cases were primarily male (67.84%) with a median age of 53 years. In general, age over 65 (OR=1.531, 95% CI: 1.432-1.637), having a contact history with animals (OR=1.286, 1.069-1.548) and having chronic diseases (OR=1.204, 1.121-1.297) are risk factors for death. However, animal contact implies a lower risk of death in the elderly. MERS mainly diffused from central Saudi Arabia to its surrounding regions, and a higher risk of disease invasion was associated with a higher coverage of bare land (hazard ratio [HR]=1.011, 95% CI: 1.002-1.020), a lower coverage of cropland (HR=0.958, 95% CI: 0.925-0.992), and a higher average temperature (HR=1.308, 95% CI: 1.205-1.420). Boosted Regression Tree (BRT) models revealed that the presence of MERS was significantly associated with the coverage of bare land, population density, elevation, a composite meteorological index and the coverage of cropland.

Conclusion: Elderly with chronic conditions are more likely to suffer from severe outcome after infection, but the role of animal contact depends on age. Coverage of bare land and cropland affected both diffusion and persistence of MERS. The risk of persistence of MERS is more related to the density of human population rather than that of camels. Future surveillance and intervention programs should target the high-risk populations and regions informed by updated quantitative analyses.

18. RESPIRATORY SYNCYTIAL VIRUS-ASSOCIATED ADULT MORTALITIES IN FLORIDA, 2014–2018

Bernhard Kloppenburg - Florida Department of Health; **Julia Munroe** - Florida Department of Health; **Heather Rubino** - Florida Department of Health; **Liza Kublalsingh** - Florida Department of Health; **Gregory Danyluk** - Florida Department of Health

Background: Respiratory syncytial virus (RSV) is an important cause of severe respiratory infections in adults ≥65 years and can exacerbate chronic underlying health conditions, resulting in increased risk for severe disease and death. Florida has a high proportion of people ≥65 years (20.5%) and has the country's longest RSV season, which provides an ideal environment to understand RSV-associated adult mortality. This study describes demographic and clinical characteristics of RSV-associated deaths in adults.

Methods: Cases were identified by querying death record data from the Florida Bureau of Vital Statistics from 2014–2018. Deaths in Florida adults that included RSV, bronchiolitis, or syncytial in the underlying/contributing combined ACME code or the initial literal causes of death were retained and counted as cases. Medical records were obtained and reviewed, and a case report form was drafted and completed.

Results: Sixty-five cases were identified. Ages ranged from 29–96 years; 86% were aged ≥65 years. White (87.69%), non-Hispanic or Latino (90.77%) females (55.38%) were overrepresented compared to Florida's population (white 77.3%, non-Hispanic or Latino 53.5%, female 51.1%). Deaths occurred in every month except June, with the highest frequency of cases expiring from December–February. Medical records were obtained and abstracted for 40 of 65 cases. Of those 40, all were

hospitalized, and the most common symptoms reported at admission were shortness of breath/respiratory distress (92.5%), cough (64.1%), or wheezing (47.5%). At least one underlying medical condition was identified in all cases. Chronic lung disease was most commonly identified (75%), followed by cardiovascular disease (67.5%) and metabolic disorders (62.5%). RSV PCR testing was performed for all 40 cases; 38 were positive. Eleven cases (27.5%) tested positive for an additional viral or bacterial coinfection, the most common being influenza and rhinovirus. Symptom onset to hospitalization ranged from 0–14 days, and hospitalization duration ranged from 1–32 days. Almost two-thirds of the cases were admitted from home and more than one-third were admitted from skilled nursing facilities.

Conclusions: Most cases of RSV-associated deaths were adults aged ≥65 years and all had pre-existing medical conditions. It is important to increase provider awareness of the potential of RSV to cause severe disease in older adults, especially in those with underling chronic lung disease, cardiovascular disease, and metabolic disorders to prompt more routine testing for RSV and to provide early interventions to prevent complications and death. This work serves as a potential baseline against which to target recommendations for future antiviral and vaccine candidates.

19. ASSESSING THE RELATIONSHIP BETWEEN WORKER IMMUNOCOMPETENCE AND SOCIAL IMMUNITY IN ACORN ANTS

Steven T. Cassidy - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Jade Chapa** - Miami Palmetto Senior High School; **Tram-Anh Tran** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Nicholas Dolezal** - Department of Biology, University of Florida; **Chelsey Gerena** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Gloria Johnson** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Arletys Leyva** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Samantha Stein** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Colin M. Wright** - Pennsylvania State University; **Carl N. Keiser** - Department of Biology, College of Liberal Arts and Sciences, University of Florida,

Eusocial insect societies possess a multi-level immune defense: the physiological immune system of individuals (immunocompetence) and colony-level protection mediated by social behaviors (e.g. corpse removal). Yet it remains unclear if and how these two levels of immune protection are related to affect the level of overall level of immune protection. Here, we examine if a relationship exists between one measure of individual worker immunocompetence, the survival after exposure to a fungal pathogen, and one colony-level immune protection behavior, the rate of corpse removal, in the acorn ant (Temnothorax curvispinosus). Results show that these colony-level and individual-level immune responses were inversely correlated such that colonies that consistently removed corpses swiftly had individuals with weaker physiological immune systems. This suggests that colony level social immune responses may partially compensate for individual immune vulnerability, and thus provide a boost in overall immune protection.

20. ASSESSMENT OF GASTROINTESTINAL PARASITES IN TWO PRIMATE SPECIES ACROSS DIFFERENT LAND TYPES IN SOUTHERN COSTA RICA

Costanza Manes - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Emerging infectious diseases are a serious threat to environmental, human and animal health. Modification of habitat and land use change are disrupting host-parasites dynamics globally. Particularly, the destruction of forests and growing human encroachment pose a risk in terms of increasing parasites transmission between different hosts and infection prevalence. Infectious diseases are often overlooked by conservation biologists. Since the coevolution of parasites and their hosts follows a density-dependent relationship, conservation policies and agenda rarely take infectious diseases control as a primary issue. However, environmental alterations are now so severe, that pathogens dynamics go beyond the assumptions of classic epidemiology. Spill-over of zoonotic pathogens poses a risk to both humans and wildlife. Thus, from ideation to completion of this study, an essential One Health approach was applied. This research investigated patterns of gastrointestinal parasites community compositions across primary pristine and secondary degraded forest landscapes, aiming at testing whether populations dwelling in secondary forests harbor different parasitic communities from populations in primary forest. This was carried out in two Neotropical nonhuman primate species, the spider monkey (Ateles geoffroyi) and the howler monkey (Alouatta palliata), living in sympatry in the Osa Peninsula, Costa Rica. A totality of 70 stool samples were collected across the selected research site and analyzed using the novel FLOTAC® parasite flotation technique. A totality of 16 parasitic morphotypes were identified. Even though no statistically significant differences between whole parasite community compositions was found, comparing singular parasitic species infection prevalence across the ecological variables revealed an interesting pattern of higher infection frequency and potential severity in secondary forest samples. This study therefore looked at which parasitic species were most recorded across the different landscapes and, throughout a review of the pertinent literature, provides probable ecological explanations behind it. Given the close phylogenetic relatedness, a high number of human parasitic zoonoses are of primate origin and easily transmit between

monkeys and humans. Control of zoonotic diseases so close to us is a complex issue, with implications for environmental, human and animal health. This project therefore highlights important findings for ecosystem health and encourages future similar studies that can shed light on the fundamental interconnection between our actions and the health of our planet.

21. CHARACTERIZING A NOVEL MURINE MODEL OF MALARIA-INDUCED PRETERM LABOR

Caitlin A. Cooper - Department of Infectious Disease, College of Veterinary Medicine, University of Georgia; **Alicer Andrew** - Department of Infectious Disease, College of Veterinary Medicine, University of Georgia; **Julie Moore** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida

Malaria infection in pregnant women is known to lead to poor maternal-fetal health outcomes, including maternal anemia, intrauterine growth restriction, pregnancy loss, and preterm delivery of low birth weight infants. The mechanisms involved in malaria-induced preterm labor are not well understood and are difficult to study in human populations. Thus, this laboratory and others have used mouse models to recapitulate some of the important pathogenic features of malaria infection during pregnancy. Here, we describe a novel murine model of malaria-induced preterm labor in C57BL/6J mice infected with Plasmodium chabaudi chabaudi AS (PccAS) at gestational days (GD) 6, 8, or 10. We observed that mice infected at all 3 timepoints experienced abrupt preterm delivery of pups, beginning on GD 16, 17, and 18, respectively. When pup viability and placenta weights were assessed a day prior to expected preterm delivery, pups from PccAS-infected dams were indistinguishable from the pups of control dams. Additional observations suggest that inflammation may not be the primary driver of preterm labor in the model. However, some preliminary evidence may support a role for oxidative stress in the placentae of infected dams to precipitate preterm labor across all three timepoints. Thus, further analyses are needed to characterize the likely mechanism of preterm labor in this model and elucidate how malaria infection contributes to this adverse outcome.

22. CROSS-SECTIONAL SURVEY OF GASTROINTESTINAL PARASITE BURDEN IN FARMED AND FREE-RANGING WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA, USA

Sydney L. Cottingham - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Heather D. S. Walden** - Department of Comparative, Diagnostic & Population Medicine; **Ramiro Isaza** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida

Gastrointestinal parasite prevalence and intensity have been described in free-ranging white-tailed deer in Florida, however, there is no published information on gastrointestinal parasite infections among farmed white-tailed deer in the state. Thus, a description of gastrointestinal parasite burden among farmed white-tailed deer is needed to inform parasite management practices and guide anthelmintic usage in Florida deer farming operations. A cross-sectional study was conducted to estimate the burden of gastrointestinal parasites in private deer farms, wildlife management areas, and agricultural research stations in Florida, and to identify host and environmental factors associated with positive diagnosis of gastrointestinal parasitism at the animal and collection site levels. Fecal samples (n=427) were collected from white-tailed deer from 5 privately-owned deer farms in north Florida (Gadsden, Liberty, Bradford, and Jefferson counties), 3 farms in central Florida (Marion, Pasco, and Sumpter counties), and 1 farm in south Florida (Hendry County). Fecal samples (n=65) and abomasum and intestines (n=25) were collected from hunter-killed deer on 6 public Wildlife Management Areas (Gadsden, Clay, Marion, Hernando, Charlotte, and Martin counties) managed by the Florida Fish and Wildlife Conservation Commission. All sample collection occurred between April 2019 and January 2020. Presence of parasite ova and larvae will be confirmed by centrifugal fecal flotation and simple sedimentation, and burden of parasite infection will be estimated from feces using modified McMasters quantification when indicated. A fluorescein-isothiocyanate-labelled peanut agglutinin assay will be used to determine the presence of Haemonchus contortus ova and larvae

among deer with trichostrongyle infections diagnosed by fecal flotation and sedimentation. Dissection of abomasum and intestine specimens will be used to enumerate and identify gross parasite specimens to species. The results of these tests will be discussed in relation to host demographics and environmental factors. These data will provide the first known description of gastrointestinal parasite burden in farmed white-tailed deer in Florida, and will identify areas for future research in gastrointestinal parasitism and parasite management practices in farmed white-tailed deer systems.

23. DERMOCYSTIDIUM INFECTION IN THE GREEN NEON TETRA PARACHEIRODON SIMULANS

Pedro H. de Oliveira Viadanna - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Worldwide, fishkeeping is an important part of the pet industry with over 1 billion individual fish traded annually. Hobbyists prefer ornamental species that are resilient under varied aquarium conditions, display vibrant coloration, and exhibit fascinating behaviors. Many small characins (family Characidae) are popular among aquarists for their vibrant coloration and have become mainstays of the freshwater ornamental fish industry. For example, members of the genus Paracheirodon are some of the most popular aquarium fishes and include the well-known cardinal (P. axelrodi), neon (P. innesi), and green neon (P. simulans) tetras. To date, diseases of characins are not well studied. This is likely because there are so many species and limited support for this line of research. However, a handful of reports have appeared involving cutaneous infections of a Dermocystidium sp. in cardinal tetras. Herein, we report infection of green neon tetras with a similar Dermocystidium sp. Fish were collected from a wild population in Brazil and exported to Florida where they were put on display for sale at a local fish store (LFS). Within a month of receipt, the LFS owners noted mucoid lesions appearing on the head, fins, and skin of some fish. The mucoid lesions were observed to expand and mature until worm-like structures appeared within the cutaneous lesions. Multiple specimens were necropsied and the worm-like structures within the mucoid cutaneous

lesions were determined by light microscopy to be Dermocystidium cysts containing spherical endospores consistent in size and shape to those previously reported in cardinal tetras. DNA extracts from the cutaneous lesions were used to amplify the 18S ribosomal DNA sequence of the Dermocystidium sp. Sanger sequencing of the resulting 1820 bp amplicon confirmed the infection was due to a Dermocystidium sp. most closely related to the Dermocystidium sp. previously reported in cardinal tetras (>99% identity). To the authors knowledge, this is the first record of a Dermocystidium sp. infecting green neon tetras.

24. ECOLOGICAL AND ENVIRONMENTAL DETERMINANTS OF SCHISTOSOMIASIS IN ETHIOPIA

Keerati Ponpetch - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Berhanue Erko** - Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia; **Teshome Bekana** - Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia; **Yang Yang** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Song Liang** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Schistosomiasis is a neglected tropical disease affecting 258 million people in 78 countries worldwide with over 90% of cases occurring in sub-Saharan Africa. In Ethiopia, schistosomiasis is caused by Schistosoma mansoni and S. haematobium with the former being the dominant species, infecting more than 4 million people and placing 35 million at risk of infection. Although many school- and community-based epidemiological surveys throughout the country have been conducted since 1960s, the national distribution of schistosomiasis endemic areas and associated environmental determinants remain little understood. The present study aims to delineate biogeographical characteristics associated with S.mansoni endemic areas and to map out potential disease risk at the national scale. Through a systematic review, we compiled a comprehensive dataset on S.mansoni surveys. A total of 95 survey sites were identified and geo-referenced, and the sites are distributed in six regional states – Southern Nations, Nationalities, and

Peoples' Region (SNNPR), Tigray, Addis, and Beneshangul Gumuz, with the majority of surveys in Amhara (52.74%) and Oromia (26.32%). The surveys exhibited a wide range of prevalence of infections from 0.43% to 95.06%. The survey sites' altitudes range from 847 to 3,141 meters above sea level, have annual mean temperatures between 17.90 – 29.80 degree Celsius, and annual cumulative precipitation between 1,400 – 1,898 millimeters. Based on landscape features, these areas are broadly classified into the following ecozones – Afroalpine, East Montane, Eastern, Northeastern, Rift Valley, Western, and West Mountain. Western Ecozone had the highest overall prevalence at 38.4%, followed by 38.14% and 34.8% in Rift Valley and East Montane Ecozones, respectively. Eight ecological niche models were developed and compared. Overall, despite local variations in the model performance, all models predicted the endemic areas in the North, West, and South of Addis Ababa, whereas Northeast and Southeast were predicted as non-endemic areas. Further, the models predicted some new areas in Illubabor Zone in Oromia and Asosa Zone in Beneshangul Gumuz to be suitable for schistosomiasis transmission. The model evaluation revealed that the ensemble model performed very well with the value of area under the receiver operating characteristic curve (AUC) at 0.958 followed by general linear models and general boosted models with AUC at 0.886 and 0.879 respectively. Our preliminary results underlined environmental and climate factors as potential drivers of the distribution of S. mansoni endemic areas and ecological niche modeling may be a useful tool for guiding the spatial targeting of disease surveillance and control interventions.

25. FIRST DESCRIPTION OF NUCLEOSPORA IN AN ELASMOBRANCH

Alisa L. Newton - Zoological Health Program, New York Aquarium, Wildlife Conservation Society; **Dalia Ferguson** - Zoological Health Program, New York Aquarium, Wildlife Conservation Society; **Anibal G. Armien** - Minnesota Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota; **Pedro H. de Oliveira Viadanna** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Cody Conrad** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Elasmobranchs are popular attractions at zoos and aquariums. In marine ecosystems they serve as apex predators and thus, are important indicators of marine ecosystem health. Sandbar sharks (Carcharhinus plumbeus) are members of the family Carcharhinidae and occur in tropical to temperate coastal waters worldwide. As part of a larger Northwest Atlantic elasmobranch study, health assessments were performed on 21 sandbar sharks captured by hook and line along the North American Mid-Atlantic coastline from 2017 to2019 during summer surveys (June – September). A blood sample was collected from the caudal vein for complete blood and differential counts, plasma chemistry and electrophoresis, and acute phase protein analysis. Examination of Wright's giemsa stained blood films identified 5 sandbar with circulating monocytes displaying karyomegaly and intranuclear spores consistent with an intranuclear Microsporidium. Formalin fixed blood samples were submitted for transmission electron microscopy, which confirmed the presence of intranuclear spores with 6 coiled filaments consistent with the microsporidian genus Nucleospora. A next-generation sequencing (NGS) approach was used to sequence DNA libraries generated from the blood nucleic acid extracts of sandbar sharks. The NGS approach recovered a 600 bp sequence that displayed >98% nucleotide identity to the18S ribosomal DNA sequence of Nucleospora salmonis that infects salmonids and Nucleospora braziliensis that infects tilapia. This sequence was used to design a nested PCR assay that confirmed the sequence was present in blood samples that displayed intranuclear spores during clinical

pathological investigations. To the authors' knowledge, this this is the first record of Nucleospora in an elasmobranch. The prevalence of the microsporidian pathogen in sandbar shark stocks and its role in disease (if any) remains to be determined.

26. FIRST REPORT OF BIPOLARIS YAMADAE LEAF SPOT DISEASE ON GUINEA GRASS IN FLORIDA

Xuechun Wang - Department of Plant Pathology, Undergraduate Research, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Ashish Adhikari** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Philip F. Harmon** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Erica Goss** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Studying fungal pathogens on weedy grasses can help fill knowledge gaps in pathogen diversity and explain the emergence of diseases of crop plants. In Florida, Guinea grass (Panicum maximum) is a common weed around agricultural crops and in other disturbed areas. Guinea grass with leaf spots resembling those caused by Bipolaris were observed in Gainesville, Florida. Isolations on Potato Dextrose Agar were made from lesions on Guinea grass leaves and the resulting fungi were identified to Bipolaris species based on morphological characteristics. DNA was extracted from mycelia and the internal transcribed spacer (ITS1 and ITS2) regions were amplified and sequenced. We identified the causal organism to be Bipolaris yamadae. Pathogenicity tests were conducted, which confirmed Guinea grass leaf spots symptoms were caused by the B.yamadae isolates. B. yamadae is known to cause leaf spots on Panicum species. The reported distribution of B. yamadae is in Cuba, Japan, China, India, Sudan, Tanzania and USA (IA, ID, ND, WI), but not in Florida. Thus, this is the first report of B. yamadae in Florida and first report of B. yamadae on Guinea grass in the United States.

27. GUT MICROBIOTA IN BEEF CATTLE AND ITS ASSOCIATION WITH ANTIMICROBIAL RESISTANCE

Peixin Fan - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lin Teng** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Zhengxin Ma** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Corwin Nelson** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Joseph Driver** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Mauricio Elzo** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Introduction: Antimicrobial resistance is a global threat to public health. Our previous study found that gut microbiota of beef cattle carries antimicrobial resistant microorganisms (ARMs), even though cattle were raised without antibiotics.

Purpose: The purpose of this study was to understand the dynamics of ARMs in the lifespan of cattle and its relationship with the development of gut microbiota.

Methods: We collected fecal samples from one generation of beef cattle (n = 278) belonging to the Angus-Brahman multibreed herd every three months throughout their lifespan, and analyzed the associations between changes in gut microbiota structure and the prevalence and concentration of cefotaxime (a third-generation cephalosporin antibiotic) resistant bacteria (CRB) in the gut.

Results: The CRB prevalence fluctuated from 10% to 40% when calves were kept on pasture, while the CRB concentration was decreased after birth ($p < 0.05$). However, when calves were relocated to the feedlot, both the CRB prevalence and concentration were dramatically increased. The major CRB isolated in the herd were Pseudomonas, Acinetobacter

and Escherichia-Shigella, which all belong to Proteobacteria. Notably, the bacterial diversity of gut microbiota was gradually increased across lifespan when calves were kept on pasture, but significantly decreased when calves were moved to the feedlot ($p < 0.05$). The Proteobacteria accounted for 50% of total meconium bacteria after birth, and decreased to 6% after three months of birth, and maintained at 2% afterwards when calves were on pasture, but increased to 6% in feedlot. Moreover, we identified several genera that belong to Firmicutes with their proportions were negatively associated with the relative abundance of Pseudomonas, Acinetobacter and Escherichia-Shigella in the gut microbial community ($p < 0.05$).

Significance: Our study indicates a role of gut microbiota in regulation of ARMs, shedding light in controlling ARMs by shaping the structure of gut microbiota.

28. INVESTIGATION OF AN UNKNOWN PYRICULARIA SPECIES ON MICROSTEGIUM VIMINEUM

Kamila Hernandez - Department of Plant Pathology, College of Agricultural and Life Sciences, University of Florida; **Brett Lane** - Department of Plant Pathology, University of Florida; **Ashish Adhikari** - Department of Plant Pathology, University of Florida; **Philip F. Harmon** - Department of Plant Pathology, University of Florida

When an invasive species establishes in a new area it gains a competitive advantage by being released from its natural pathogens. With time, pathogens may emerge on the invasive species and the competitive advantage may change. The Big Oaks National Wildlife Refuge in Madison, Indiana is home to native plant species and non-native plant species such as the Microstegium vimineum. Multiple fungal pathogens have recently emerged on M. vimineum. In the summer of 2018, a new disease was observed on M. vimineum at our Big Oaks National Wildlife Refuge field site. The objective of this research was to identify the pathogen causing this new disease on M. vimineum. Symptomatic leaves exhibited diamond to elongated lesions isolated to the leaf tissue. A Pyricularia-like species was successfully isolated from 13 leaves. Conidia were pyriform with 2-3 cells per conidia. Conidia are 15.16-16.97 µm in length (16.24 µm average) and 5.65-8.99 µm in width (7.53 µm average). We sequenced the internal transcribed spacer to identify the isolates.

Comparison to sequences in the NCBI GenBank database revealed no matches with greater than 93% identity. Phylogenetic analysis revealed the pathogen as a previously uncharacterized Pyricularia-like species and may represent a new genera. This finding will contribute to ongoing research on the effects of emerging pathogens on invaded plant communities.

29. INVESTIGATION OF THE EXTRINSIC PATHWAY OF COAGULATION AS A THERAPEUTIC TARGET FOR PLACENTAL AND CEREBRAL MALARIA

Tara C. Bracken - University of Georgia; **Alicer Andrew** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Caitlin A. Cooper** - University of Georgia; **Tiffany Jenkinson** - University of Georgia; **Tamas Nagy** - University of Georgia; **Benjamin Brainard -** University of Georgia; **Nigel Mackman** - University of North Carolina - Chapel Hill; **Julie Moore** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Sequestration of Plasmodium falciparum-infected erythrocytes in the brain or the maternal blood space of the placenta results in two severe clinical syndromes, cerebral (CM) and placental malaria (PM), respectively. A key feature of both is hypercoagulation and fibrin deposition. Here, a combination of genetic and therapeutic approaches were employed to investigate the importance of expression of Tissue Factor, a potent initiator of the extrinsic coagulation cascade, and efficacy of anticoagulant treatment, in experimental mouse models for both syndromes. For experimental cerebral malaria (ECM), virgin C57BL/6J (B6) mice, mice with floxed TF (F3fl/fl) and transgenic for cre recombinase under control of the Tie2/Tek promoter (TFdeltaEc) or Lysozyme M promoter (TFdeltaMy), and “low TF mice” (LTF) mice that are completely deficient in mouse TF and are transgenic for human TF were infected with 106 P. berghei ANKA, a virulent murine malaria species capable of inducing ECM. For experimental placental malaria (EPM) impregnated mice of the same strains were infected with 106 P. chabaudi AS, a nonvirulent parasite strain which is typically spontaneously cleared by mice on a B6 background but causes pregnancy loss. The results reveal that treatment of B6 ECM mice with a low molecular weight heparin (LMWH), which targets coagulation Factor X and thrombin downstream

of TF, improves outcomes in ECM. Moreover, the results in LTF mice show that both neurological impairment and vascular damage associated with ECM are attributable to TF as LTF mice had very low levels of both indicators. In EPM, both LMWH treatment and depletion of TF on endothelium in TFdeltaEc mice resulted in significantly improved pregnancies outcomes. Thus, dysregulated TF function contributes to pathogenesis in both ECM and EPM, implying that anticoagulant therapies are appropriate adjunctive therapies to consider in human CM and PM.

30. PLACENTAL MALARIA INDUCES OXIDATIVE STRESS IN HUMAN SYNCYTIOTROPHOBLAST

Demba Sarr - University of Georgia; **Brittany Russ** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Jeffrey Ecker** - University of Georgia; **David S. Peterson** - University of Georgia; **Julie Moore** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Placental malaria is characterized by accumulation of Plasmodium falciparum-infected erythrocytes and maternal inflammation in the intervillous space of the placenta. These features, in particular the latter, are associated with placental damage and fetal compromise. However, understanding of the mechanisms that lead to poor pregnancy outcome and interventions targeting excessive host responses to placental malaria are still lacking. The syncytiotrophoblast, a cell of fetal origin, is known to be responsive to malaria-infected erythrocytes as well as the malaria toxin, hemozoin, but its susceptibility to oxidative stress and how this might contribute to placental damage and dysfunction has not yet been directly investigated. The characteristics and key drivers of the syncytiotrophoblast response to oxidative stress were investigated using ex vivo human placental tissues and primary trophoblasts isolated from healthy pregnant women. Primary syncytiotrophoblast was exposed to hemozoin and tumor necrosis factor, a critical inflammatory cytokine, to model conditions found in pathogenic placental malaria. The data show remarkable lipid peroxidation in human placental samples from a malaria endemic setting and increased markers of an anti-oxidative response and oxidative damage in syncytiotrophoblast exposed to hemozoin, tumor

necrosis factor, and tumor necrosis factor combined with hemozoin. These results suggest that oxidative stress may be a key driver of trophoblast functional compromise in placental malaria and could be targeted therapeutically to mitigate the poor outcomes associated with this syndrome.

31. RELATIONSHIP BETWEEN IMPROVED SANITATION AND SOIL TRANSMITTED HELMINTH INFECTIONS AMONG CHILDREN IN PERI-URBAN MAPUTO, MOZAMBIQUE

Lina Babiker - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Yi Su** - Institute for Child Health Policy, College of Medicine, University of Florida; **Joseph Brown** - Department of Environmental Engineering Sciences, Georgia Institute of Technology; **Oliver Cumming** - Department of Environmental Engineering Sciences, London School of Hygiene & Tropical Medicine; **Yang Yang** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Song Liang** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Increased urbanization in low- and middle-income countries introduces new threats to public health as individuals move from less dense rural areas to highly populated, informal, urban slums. Closer living quarters accompanied with a lack of appropriate water, sanitation, and hygiene puts children in these populations at an increased risk of gastrointestinal infections. Chronic soil transmitted helminth (STH) infections are of particular concern in children as they have been associated with poor health outcomes including malnutrition, stunting and developmental delays. In order to measure the effects of improved sanitation on child health in these increasingly common environments the MapSan study, a two-year before-and-after with control trial, was conducted in peri-urban Maputo, Mozambique. 537 compounds across 16 neighborhoods with 1,731 children under five were divided into intervention and control arms of the sanitation trial. The intervention consisted of the construction of communal sanitation blocks in larger compounds and shared latrines in smaller compounds. All household members in both study arms were offered a broad-spectrum deworming treatment after improved

sanitation installation. Baseline, midline, and final detailed health surveys and stool samples were collected 12 months prior to intervention implementation, at the time of implementation, and 12 months post implementation. Initial analysis of this data shows an increase in the prevalence of overall STH infections for children in control compounds across all surveys (49.23% at baseline, 50.47% at midline, and 51.00% at final). Children in intervention compounds had a slight decrease in STH prevalence between midline and final surveys (47.91% at baseline, 49.80% at midline, and 49.14% at final). A spatially explicit risk assessment approach is utilized to explore children's STH re-infections and associations with sanitation and treatment.

32. SURVEILLANCE FOR DRUG RESISTANCE IN CANINE HOOKWORMS IN NORTHERN FLORIDA SHELTERS

Morgan D. Weldon - College of Veterinary Medicine, Department of Comparative, Diagnostic, and Population Medicine, University of Florida; **Daniela M. Maura** - College of Veterinary Medicine, Department of Comparative, Diagnostic, and Population Medicine, University of Florida; **Lawrence N. Garcia** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Alice C. Y. Lee** - College of Veterinary Medicine, Department of Comparative, Diagnostic, and Population Medicine, University of Florida

The hookworm, Ancylostoma caninum, is the most common intestinal helminth of dogs in the United States, particularly in the southeast. Infection can lead to anemia and also poses a public health risk as immature hookworms can cause itchy skin lesions in people. Multi-drug resistant A. caninum isolates have recently been confirmed in a number of dogs from Florida. Therefore the purpose of this study was to determine whether resistance to pyrantel, a commonly used anthelmintic drug, is present in A. caninum in northern Florida shelter dogs. Fecal samples collected from shelter dogs were examined by sugar centrifugal flotation for A. caninum eggs. Dogs were treated with pyrantel according to the shelter's normal dosing protocol. Post-treatment fecal samples were collected 6-11 days later from dogs that previously tested positive for A. caninum. Egg counts were performed on all pre- and post-treatment samples using the Mini-FLOTAC device in order to calculate the fecal egg count reduction (FECR) after treatment. FECR < 90% was

considered a sign of A. caninum resistance to pyrantel. Of 48 dogs in the study, only 11 dogs were successfully cleared of infection (i.e. FECR of 100%). Mean FECR in this group of dogs was 74.1%, with a 95% confidence interval of 63.8-84.4%. These results suggest the presence of pyrantel-resistant A. caninum in the northern Florida dog population, and should prompt careful monitoring of infection status and confirmation of parasite clearance prior to adoption.

33. FACTORS ASSOCIATED WITH SELF-REPORTED TUBERCULOSIS (TB) IN PERSONS LIVING WITH HIV IN FLORIDA

Nana Ayegua Hagan Seneadza - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Zhi Zhou** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Babette Brumback** - Department of Biostatistics, College of Public Health and Health Professions, University of Florida; **Michael Lauzardo** - Emerging Pathogens Institute, College of Medicine, University of Florida; **Awewura Kwara** - Emerging Pathogens Institute, College of Medicine, University of Florida; **Cindy Prins** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Nicole Ennis** - Florida State University; **Jamie Morano** - University of Southern Florida; **Marie Nancy Seraphin** - Emerging Pathogens Institute, University of Florida; **Robert Cook** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida,

Introduction: Tuberculosis (TB), a major public health problem, is one of the most common opportunistic infections and the leading infectious cause of death in persons living with HIV/AIDS (PLWH). The identification of factors associated with TB infection or disease (TB) status could provide useful insights into the risk of TB among PLWH. This study assessed factors associated with self-reported TB status in PLWH in Florida. We hypothesized that sociodemographic, behavioral and health status characteristics are associated with self-reported TB.

Methods: We analyzed survey data of 880 PLWH recruited from HIV clinics and surrounding communities in Florida. We used chi-square analysis and multivariable logistic regression models to determine factors associated with self-reported TB. Self-reported TB was measured as a binary (“Yes” and “No”) outcome variable based on the responses to the question “Have you ever been diagnosed with TB, or been told you have a positive skin test (sometimes called a PPD) or a positive TB blood test

(called a Quantiferon Gold or T-spot test)?" Significance level was set at p-value ≤ 0.05.

Results: The prevalence of self-reported TB was 15.9% (140 out of 880). Using Chi-square analysis, age ≥ 45 years, being Black/African American, being homeless, and non-injection crack cocaine use were significantly associated with self-reported TB. In the multivariable logistic regression, being Black/African American and age ≥ 55 years remained significantly associated with self-reported TB, with adjusted odds ratios (aOR) of 2.37 (95% CI: 1.40-4.02) and 2.24 (95% CI: 1.07-4.67) respectively after controlling for other variables such as homelessness and non-injection crack cocaine use.

Conclusion: The prevalence of self-reported TB in this sample of PLWH is comparatively higher than national estimates of TB infection or disease. Among PLWH in Florida, being Black/African American and aged ≥ 55 years were strong predictors of self-reported TB status. Self-reports could provide useful insights on factors associated with TB in PLWH especially when other sources of information are unavailable. A comparison of self-reported TB with TB diagnosis in medical records and surveillance data using this same population is needed to determine the agreement between these sources of information.

34. RECOMBINANT ATTENUATED SALMONELLA VACCINE PRODUCING MYCOBACTERIUM TUBERCULOSIS ANTIGENS: A NOVEL IMMUNOMODULATOR

Hedwig Kitdorlang Dkhar - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Sameeksha Alva** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Christie Hay** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Shilpa Sanapala** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Shifeng Wang** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Mike Mathieu** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Roy Curtiss III** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Josephine Clark-Curtiss** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Tuberculosis (TB) continues to be one of the top 10 causes of death worldwide, responsible for more deaths in humans than any other disease caused by a single microbe: 1.5 million people in 2018. The limitations of and the short-term protection afforded by the only globally approved vaccine, Mycobacterium bovis Bacille Calmette-Guérin (BCG) has prompted researchers worldwide to develop better and more efficacious vaccines that elicit improved innate and adaptive immune responses against Mycobacterium tuberculosis. We have developed Recombinant Attenuated Salmonella Vaccines (RASVs) to deliver candidate M. tuberculosis antigens to elicit better humoral and cell-mediated immune responses against M. tuberculosis antigens than BCG or other candidate vaccines. In our studies, we found that RASV χ12068(pYA4891, specifying synthesis and secretion of ESAT-6, CFP-10 and Ag85A) gave better protection than any other RASV tested and at least equivalent to BCG. To better understand the mechanisms of protection, we evaluated antigen-specific antibody (IgGs) titers in sera and CD4+ and CD8+ T-cell responses in lungs and spleens of immunized mice. We found that χ12068(pYA4891) elicited higher levels of total IgG to Ag85A, ESAT-6 and CFP-10 than BCG and that the major isotype of the antibodies to each of these antigens was IgG2b. Using purified protein

antigens or NIH-synthesized tetramers for ESAT-6 and CFP-10, we found better T-cell responses/expansion in the lungs and spleens of RASV-immunized mice compared to BCG-immunized mice, especially with regard to CD8+ T-cell responses. We also evaluated the protective role of two additional candidate Mtb antigens, FAP/Apa (Fibronectin attachment protein/apa) and TB15.3 (a universal stress protein) when they were delivered by RASVs in combination with ESAT-6, CFP-10 and Ag85A and found that both antigens enhanced protection of RASV-immunized mice. Our data demonstrate the advantages of using RASVs delivering multiple (up to 5) M. tuberculosis antigens as immunomodulators that activate host immunity to protect against M. tuberculosis infection.

35. A SURVEY OF TICK-BORNE BACTERIAL AND VIRAL PATHOGENS IN FLORIDA

Carrie De Jesus - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **William H. Kessler** - Department of Geography, University of Florida; **Claudia Ganser** - Department of Geography, Emerging Pathogens Institute, University of Florida; **Chanakya Bhosale** - Department of Entomology and Nematology, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Gregory Glass** - Department of Geography, Emerging Pathogens Institute, University of Florida

Within the past 3 decades, new bacterial and viral etiological agents of tick-borne disease have been discovered in the southeastern US and the number of reported tick-borne pathogen infections have increased. In Florida few systematic studies have been conducted to determine the presence of tick-borne pathogens. This investigation examined the distribution and presence of tick-borne bacterial and viral pathogens in Florida. Ticks were collected by flagging at 41 field sites, across 28 counties in mainland Florida from 2016-2019. DNA and RNA were extracted individually from 1,757 ticks. We screened for the following pathogens: Anaplasma, Borrelia, Ehrlichia, Rickettsia, Powassan virus, Heartland virus and Bourbon virus. Bacterial pathogens were screened for using conventional PCR methods with previously established primer sets. Viral pathogens were tested using previously established q-PCR primer probe sets. Four species of ticks were collected: A. americanum, A. maculatum, D. variabilis and I. scapularis. Within these ticks, 6 bacterial species were identified: B. burgdorferi, B. lonestari, E. ewingii, R. amblyommatis, R. andeanae, R. parkeri and Rickettsia endosymbionts. Rickettsia amblyommatis was the most prevalent (29%) pathogen detected in the most collected tick, Amblyomma americanum. Pathogenic Borrelia, Ehrlichia and Rickettsia species were all detected in the north

and north-central Florida counties. All other bacterial and viral pathogens had low or undetectable prevalence's (0-1.4%). Given the diversity and numerous bacterial species detected in ticks in Florida, further investigations should be conducted to identify regional hotspots of tick-borne pathogens, especially those associated with Amblyomma americanum.

36. APPLICATION AND MODELING OF A TICK-KILLING ROBOT

Alexis White - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Holly Gaff** - Department of Biological Sciences, Old Dominion University

Ticks are vectors of disease-causing pathogens that affect humans, wildlife, and domestic animals. Effective control measures are needed to reduce the risk of encountering ticks, and thus reduce risk of tick-borne disease. To control ticks, a variety of methods have been used including the more recent invention TickBot, a tick-killing robot. TickBot lures ticks, using movement and carbon dioxide, to a permethrin treated cloth as it circles a predetermined perimeter. Previous studies have shown TickBot's ability to protect a treated area from tick encounters for approximately 24 hours. Mathematical models can be used as part of the One Health approach to explore tick population dynamics, quantify risk of tick-borne disease, and identify strategies to reduce that risk. Using the data from the ODU Tick Research Team surveillance project, along with the results from TickBot field studies, a mathematical model was developed to explore ideal usage of the TickBot as an integrated tick management tool. Results from the model will be presented and discussed.

37. ASSESSING HOW THE MICROBIOME IMPACTS NEUROLOGICAL SEQUELAE OF VENEZUELAN EQUINE ENCEPHALITIC VIRUS (VEEV) IN PANAMA

Lauren Aycock - College of Medicine, University of Florida; **Jean Paul Carrera** - Gorgas Institute; **Davis Beltran** - Gorgas Institute; **Edgardo Abadía** - Gorgas Institute; **Josefrancisco Galue** - Gorgas Institute; **Joan Whitlock** - Department of Infectious Disease, College of Medicine, University of Florida; **Gary Wang** - Department of Infectious Disease, College of Medicine, University of Florida; **Amy Vittor** - Department of Infectious Disease, College of Medicine, University of Florida

Venezuelan equine encephalitic virus (VEEV) is a mosquito-borne virus endemic to Darién, Panama that can result in long-lasting neurological sequelae months after clearing the acute encephalitic infection, including paralysis, sensory deficits, recurrent headaches, fatigue, depression, and memory loss. Substantial evidence has demonstrated that the microbiota-gut-brain axis is significantly associated with neurodegenerative disease, and the link between obesity, microbiome dysbiosis, and neuroinflammation suggests the Panamanian population may be at an increased risk of neurological sequelae secondary to encephalitic viruses. The goal of this study is to demonstrate a link between the over/underabundance of certain bacterial species in the microbiome and the onset of neurological sequelae in individuals previously infected with encephalitic disease in Darién, Panama, in order to better understand the pathogenesis of these arboviruses, as well as shed light on the intersection between chronic and infectious disease. Working with a team of epidemiologists from the Gorgas Institute, over 200 sera samples were collected from individuals in the community to test for the presence of antibodies to VEEV and other encephalitic viruses. Surveys were conducted to assess symptoms of an acute encephalitic infection (e.g. fever, headache, malaise, etc.) or neurological sequelae after resolution of the infection (e.g. memory loss, confusion, impaired activities of daily living, etc.). In addition, 39 human stool samples were collected in the field and DNA was extracted in-country from 34 of these samples. The samples are pending analysis at the University of Florida. Future studies will focus on performing 16s rRNA sequencing on the DNA extracted from the stool samples in order to assess the prevalence of specific bacterial species. The sera samples will

be analyzed to look for markers of chronic inflammation. The bacterial prevalence, inflammatory markers, and symptomatic survey data will be analyzed to search for correlations between microbiome and neurological sequelae. Current neurodegenerative theory suggests the microbiota-gut-brain axis may augment neuroinflammation, resulting in neurological sequelae following exposure to encephalitic viruses. By studying the microbiome of exposed individuals in Darién, it may be possible to find a connection between chronic health and the prevention of neurologic sequelae.

38. ASSESSING THE IMPACT OF HYPOXIC CONDITIONS DURING IRRADIATION ON LONGEVITY AND STERILITY OF AEDES AEGYPTI FOR USE IN STERILE INSECT TECHNIQUE

Dylan Tussey - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Daniel Hahn** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

Ionizing radiation from x-rays, gamma rays, and electron beams are used very efficiently to sterilize insects by fragmenting their genomic DNA as part of sterile insect technique programs (SIT). However, radiation exposure can also induce a range of off-target effects that reduce the overall quality and performance of sterilized males. Oxidative stress to critical somatic cellular components is believed to cause many of these off-target effects. Subjecting insects to hypoxia before and during irradiation has been found to reduce oxidative stress and improve performance of sterilized males of some insect species, but hypoxia may also preserve fertility. The objective of this study was to determine the extent to which hypoxic treatments prior to and during irradiation can induce a hormetic response in male Aedes aegypti mosquitoes prior to irradiation. Male Ae. aegypti pupae were exposed to 1 h of hypoxia by being placed in sealed containers and flushed with nitrogen to determine effects of hypoxia on longevity and mating performance when exposed to radiation at doses of 0, 20, 50, 65, 85, or 100 Gy. Survival was assessed daily to compare longevity among treatments. Subsets of males were caged individually with 3 wild-type females for assessment of mating performance and sterility. Hypoxia treated males had shorter longevity and performed worse than control males at low radiation doses but had

similar or increased longevity than control males after exposure to higher radiation doses. Males irradiated in hypoxic conditions did retain some fertility at higher doses of radiation.

39. ASSESSING THE USE AND REJECTION OF RABIES POST-EXPOSURE PROPHYLAXIS – LEE COUNTY, FLORIDA, 2016–2018

Alexis Bell - Florida Department of Health; **Jennifer Roth** - Florida Department of Health

Background: Rabies is a major health concern following an animal exposure despite being preventable through timely administration of rabies post-exposure prophylaxis (rPEP). The Advisory Committee on Immunization Practices (ACIP) has published guidelines for administration of rPEP. The extent to which rPEP recommendations and usage are consistent with these guidelines is not well known. All cases with possible rabies exposures and any case where rPEP is recommended are reportable in Florida. This analysis sought to characterize the epidemiology of animal exposures in Lee County, Florida and identify patterns among cases and rPEP usage.

Methods: All possible rabies exposure cases reported to the Florida Department of Health in Lee County (Lee CHD) from January 3, 2016 to December 29, 2018 were assessed. Exposures for which rPEP was recommended or initiated were retrieved from Merlin, Florida's electronic disease reporting system. All rPEP cases were reviewed and categorized by the agency and reason rPEP was recommended.

Results: There were 4,816 possible rabies exposures reported during the study time frame. Of these, 558 were recommended or initiated rPEP. There was a significant increasing trend (p=0.037) in the rate of rPEP cases over the three-year time period. Exposures involved dogs (63%), cats (24%), and wildlife (12%). The animals involved were stray (42%), owned (37%), and wild (11%). Although rPEP was recommended for 539 cases, only 36% initiated it. rPEP was most frequently rejected when the exposure was to a dog (n=210, 75%), an owned animal (n=145, 52%), the animal was not found/escaped (n=134, 48%), or recommended by Lee CHD (n=267, 95%). The top three reasons for rPEP recommendations were: animal was not found/escaped (n=321), report had missing owner information (n=74), and the victim could not be contacted (n=66). There

were 18 cases in which rPEP was initiated when not recommended, primarily involving dogs, cats, and small rodents. In 14 cases, rPEP was recommended by the ED/hospital, and in six cases the animal was reported healthy after the 10-day observation period.

Conclusions: Lee CHD saw an increase in the rate of rPEP cases between 2016–2018. rPEP was often recommended due to non-responsiveness or a lack of information and had low uptake among cases with lower risk for rabies. Investigations are necessary to rule out risk of rabies infection in humans but can be time consuming for staff. Finding ways to increase responsiveness and data quality to improve risk assessment can relieve some burden and ensure rPEP is being used efficiently.

40. BLUETONGUE VIRUS SURVEILLANCE AMONG FARMED DEER IN FLORIDA

Caroline Stephenson - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Julia C. Gibson** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Karly A. Caples** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Olivia Goodfriend** - Department of Wildlife Ecology and Conservation, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Emerging Pathogens Institute, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Bluetongue viruses (BTV) belong to the genus Orbivirus and the family Reoviridae. There are twenty-seven currently known serotypes, and novel types continue to emerge. BTV can infect wild and domesticated ruminants and bovines. Disease presentation in sheep and deer can be severe and lead to death. Common symptoms of bluetongue disease include fever, swelling of mouth tissues, lameness, difficulty swallowing

and hemorrhage. As such, infections with BTV are responsible for considerable economic losses globally. Culicoides spp. biting midges are the vector of BTV. The Cervidae Health Research Initiative (CHeRI) seeks to increase the health and production of farmed deer by conducting surveillance for common and novel pathogens affecting cervid populations in Florida (FL). It is under this research initiative that we screen tissues from deceased farmed deer in FL for BTV to understand the burden of this virus. Field technicians deploy to farms when a deer has died, and perform a necropsy. They save key deer specimens, including spleen tissue. We then homogenize a portion of the spleen tissue and extract virus genomic RNAs (vRNAs). We utilize a pan-BTV real-time reverse transcription polymerase chain reaction (rt RT-PCR) assay to detect vRNA from known BTV serotypes. However, current understanding of which serotypes are circulating in FL is limited. We are therefore developing serotype-specific assays to determine which BTV serotypes are responsible for deer infections between 2016 through the present. Thus far, there have been 89 BTV-positive deer, and the virus in nine of those samples typed using the assays we developed, and one through next-generation sequencing. The viruses were identified as: one BTV-1, one BTV-3, three BTV-10, one BTV-11, one BTV-17, and three BTV-18. Our results provide the most current data regarding the circulation of BTV serotypes in FL, and reveal that three serotypes not typically present in the state (BTV-1,-3, -18) are now affecting farmed deer in Florida. The assays we developed are sensitive and specific for the detection of BTV serotypes -3, -10, -11, -17 and -18 and we have several more assays in development. This work is furthering our understanding of the impact the BTV on cervids in FL. Lastly, the genome sequences from isolated BTVs are being sent to collaborators who are developing a vaccine against BTV for farmed deer, so our work is crucial to determine which serotypes are of most concern and should be covered in the vaccine.

41. CD44 IS A FUNCTIONALLY RELEVANT RECEPTOR FOR ADHERENT PLASMODIUM FALCIPARUM IN THE PLACENTA

Simon O. Owino - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Julie Moore** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Plasmodium falciparum infected red blood cells (iRBCs) accumulate in the maternal blood space of the placenta during malaria infection, culminating in pathological consequences deleterious to pregnancy success. The fetal cell in contact with maternal placental blood is a syncytialized epithelium called syncytiotrophoblast (ST). The placenta has a rich supply of low sulfated chondroitin sulfate A (CSA), a principle ligand for VAR2CSA parasite protein, present on the surface of placenta-adherent iRBCs, but the critical proteoglycans bearing CSA that participate in placental adherence and influence the course of infection have not been studied. Given that ST is immunologically active in the presence of iRBCs, here we examined the role of CD44 proteoglycan, a known CSA bearing molecule with a trans-membrane cytoplasmic domains adept at signaling functions, in iRBC/ST functional interactions. Using a flow cytometric approach with extracted ST proteins, we show specific CD44 protein binding on the surface of CSA-adherent iRBCs, an interaction that is dependent on CSA, since chondroitinase treatment of syncytiotrophoblast membrane proteins (SMPs) significantly reduced binding and a parasite line devoid of VAR2CSA did not capture CD44. Lentiviral knockdown of CD44 expression in the choriocarcinoma cell line, BeWo, confirms a significant role for ST CD44 in iRBC binding. Additionally, we show by western blot CD44-dependent changes in tyrosine phosphorylation status of a series of ST proteins and confirm involvement of Src Kinases using specific inhibitor, indicating a potential functional role for CD44 in the ST immunological response. By using a specific Src kinase inhibitor, we confirm involvement of this family of kinases in response to iRBC activation. Furthermore, malarial exposure appears to enhance CD44 expression by ST cells in vitro as assessed by protein detection in cell lysates by ELISA. In summary, we provide evidence for CD44 proteoglycan potential dual role as an in vivo receptor for VAR2CSA expressing iRBCs as well as an ST signaling molecule modulated by malaria infection.

42. CHARACTERIZATION OF PLASMODIUM PERFORIN-LIKE PROTEIN-3 (PPLP3) FUNCTION DURING OOKINETE INVASION OF THE MOSQUITO MIDGUT

Prachi Khare - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Borja López-Gutiérrez** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Vincent Nyasembe** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Timothy Hamerly** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Malaria is a devastating disease with 450,000 deaths reported in 2017, primarily in children under 5 years of age. Malaria is caused by Plasmodium parasites, which are transmitted by its obligate invertebrate vector, the Anopheles mosquito. Malaria transmission-blocking vaccines (TBVs) are a promising approach in efforts towards eradicating malaria, as TBVs disrupt the ability of the Plasmodium ookinete from establishing an infection in the mosquito. The Anopheles Alanyl aminopeptidase N-1 (AnAPN1) is mosquito midgut receptor that has been demonstrated to be essential for transmission of Plasmodium parasite through the mosquito and is currently under development as a TBV target. While antibodies raised against AnAPN1 are effective at preventing ookinete traversal of the midgut, the precise mechanism of AnAPN1 interaction with parasite ligands are unknown. We posit that traversal of the midgut epithelium by the ookinete stage of the parasite is mediated by a direct interaction of ookinete proteins with AnAPN1 on the apical layer of the mosquito midgut. Known binding proteins for midgut APN1 receptors in other insects include crystal (Cry) proteins, which are pore forming endotoxins. Plasmodium Perforin-like Proteins (PPLP) -3, - 4 and -5 are parasite secreted proteins, predicted to have a similar function to Cry proteins in mosquitoes and play important roles in ookinete invasion of mosquito midgut. Previous studies show that knockdown of PPLP-3 completely blocks oocyst formation, as compared to knockdowns of PPLP-4 and -5, which still result in very low numbers of oocysts, indicating a more important role of PPLP-3. We hypothesize that PPLP-3 is a parasite

interacting protein that interacts with AnAPN1. PPLP-3 and AnAPN1 interaction initiates the formation of a pore on the apical layer of the midgut epithelium, enabling the ookinete to invade the midgut epithelial cell and traverse to the basal side to form an oocyst. To study this interaction, we will utilize the AlphaLISA technique to measure direct interaction between Plasmodium falciparum-expressed haemagglutinin (HA)-tagged PPLP-3 and full-length, enzymatically active AnAPN1. To this end, we are generating a transgenic P. falciparum (3D7) parasite line expressing HA-tagged PPLP-3 ectopically in asexual stages. We also propose to confirm if pores are formed on Anopheles gambiae mosquito midgut sheets by PPLP-3, -4 and -5 by immuno- electron microscopy utilizing antibodies specific to PPLP-3, -4 and -5. The overarching goal is to elucidate the role of PPLP-3 in ookinete invasion of mosquito midgut and provide insight into the mechanism transmission-blocking activity of anti-AnAPN1 antibodies.

43. CHARACTERIZATION OF SPATIAL AND TEMPORAL FACTORS RELATED TO EASTERN EQUINE ENCEPHALITIS VIRUS SPILLOVER IN ORANGE COUNTY, FLORIDA

Stephanie J. Mundis - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Sadie Ryan** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Eastern equine encephalitis virus (EEEV) is a highly pathogenic mosquito-borne viral infection that affects both humans and horses, with a high case fatality rate and long-lasting, debilitating sequelae associated with human infections. This virus is primarily vectored between Culiseta melanura and various bird species, although Culex erraticus is thought to play a role in maintenance of EEEV transmission in Florida. In Orange County, Florida, vector control professionals use sentinel chicken flocks to identify the timing and location of EEEV transmission. In this project, we assessed spatial and temporal factors for associations with vector abundance and EEEV detection. We first tested for associations between the land cover composition and configuration and vector species abundance, before comparing EEEV-positive to EEEV-negative sentinel chicken flocks for 2013 in the county. Additionally, we used cross correlation coefficients to test for significant associations between

meteorological factors and vector abundance at multiple temporal lags, finding that temperature four weeks prior to the collection date and precipitation approximately eight weeks prior to the collection date were positively associated with county-wide Culiseta melanura and Culex erraticus abundance. The results from these analyses will comprise the basis for further modelling aiming to predict the timing and location of EEEV spillover in the context of Orange County.

44. CLINICAL AND EPIDEMIOLOGICAL PROFILE AMONG BLOOD DONORS WHO SCREEN POSITIVE FOR CHAGAS DISEASE IN MARICOPA COUNTY, ARIZONA, 2007-2018

Norman L. Beatty - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Kaci McCoy** - Emerging Pathogens Institute, University of Florida; **Melissa Kretschmer** - Maricopa County Department of Public Health, Disease Control Division, Office of Epidemiology

Chagas disease is a neglected tropical disease caused by the kinetoplastid protozoan parasite, Trypanosoma cruzi. In 2007, voluntary screening of blood donors for serologic evidence of Chagas disease was initiated throughout the United States after recognition of transfusion-related infection. In the state of Arizona, Chagas disease is a mandatory reportable infectious disease to state and local public health departments. Maricopa County Department of Public Health in Phoenix, Arizona, has been notified of any positive blood donor with detection of screening antibodies to Trypanosoma cruzi among those who live within the county since 2007. When a person screens positive for Chagas disease after blood donation they typically receive a letter from the blood donor servicer. We analyzed retrospective data on all reported positive blood donors to conduct a cross-sectional analysis assessing demographic information and environmental exposures, travel history, Triatoma species (kissing bug) exposure and bite history, and symptoms of Chagas disease were collected when available. Thirty-five individuals tested positive for Chagas disease from 01/02/2007 to 12/31/2018. Median age 40.5 years, 57% male, and 77% Hispanic or Latino ethnicity; five individuals were 18 years or younger and 80% of females were of child bearing age. Eight people (23%) were lost to follow-up and unable to be reached for interview with an epidemiologist. Majority who tested

positive had lived or were born in Latin America (N=24/27; 88%) with Mexico being the predominant country of origin (Mexico: N=23/24 (95%); Argentina: N=1/24 (5%). States traveled to in Mexico (may be more than 1 location per individual): N=Sonora 5/23; Michoacan N=2/23; Jalisco N=3/23; Zacatecas N=3/23; Guerrero N=2/23; Guaymas N=2/23; Queretaro N=1/23; Chihuanhan, N=1/23; Baja California N=1/23; Quintana Roo N=1/23; "Southern Mexico" N=2/23; unknown state N=1/23. At the time of questioning, twenty-three were asymptomatic (N=23/26; 87%) and one individual deferred to answer these questions. Two individuals had symptoms consistent with Chagas disease (one patient with megacolon and intestinal dysfunction, one patient with chronic fatigue), and one patient had died from sudden cardiac arrest due to cardiomyopathy (previous pacemaker in place) when speaking to family. Only four positive blood donors had confirmatory testing sent to the Centers for Disease Control and Prevention, with N=2/4 (50%) being confirmed positive. None of those who tested positive via blood donation for Chagas had received antitrypanosomal therapy. Blood donor screening for Chagas disease can help identify possible cases of infection but more awareness and resources are needed once a person has screened positive to ensure for appropriate linkage to care, and further management if Chagas disease is confirmed.

45. DECLINING FORCE OF INFECTION OF DENGUE IN THAILAND AND ITS CLINICAL IMPLICATIONS

Angkana T. Huang - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Saki Takahashi** - University of California, San Francisco; **Isabel Rodriguez-Barraquer** - University of California, San Francisco; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Age of reported dengue infections in Thailand has continuingly increased since the 1980s. Suspected factors for the increase includes demographic transition, urbanization, vector control efforts, and changes in reporting. Despite the unknown degree to which each of these factors contributes, past modelling efforts which incorporates various subsets of them consistently estimated overall trending declines in force of infection (FoI) throughout the country. However, one critical aspect that remained unexplored is the clinical detectability of dengue infections. Being infected with one of the four human circulating serotypes of dengue confers life-long immunity to the infecting serotype and a period of heterotypic cross-protection followed by increased risk for severe disease in subsequent infections due to antibody dependent enhancement (ADE). The decline in FoI implies lengthening intervals between the infections, a factor shown to be correlated with the amplitude of ADE. Hence, it becomes unclear if the clinical detectability remains constant, and if not, how does that affect the FoI estimates. Age-varying FoIs, time- and age-varying reporting rates, clinical fractions for each of the four probable infections, and the rate of change of those fractions were simultaneously estimated from provincial age-stratified dengue case counts from 1981-2017 and their underlying population age structure using Hamiltonian Monte Carlo Markov Chains (MCMC). Performance was compared against nested subsets of the model to avoid over-parameterization. Accounted for changes in reporting rates and changes in clinical detectability of the infections, declines in FoI remains observed. Compared to the nested models, the full model showed equivalent if not superior performance in most provinces suggesting non-negligible importance of these factors to the increasing age of reported cases phenomena. 95% credible intervals for rate of change in clinical fractions excluded zero in the majority of the provinces. Infection histories reconstructed from these estimates shows

that newer birth cohorts were experiencing longer waiting times to infections than in the past and at lower synchrony. Statistical modelling, with explicit adjustments of age variations in FoIs and age- and time- time varying reporting rates, supports the change of clinical manifestations of dengue in Thailand over the study period. The change coincides with lengthening intervals between infections and increased infection asynchrony which implies being infected with dengue strains that are more dissimilar genetically and perhaps antigenically. Further studies are needed to determine whether the observations are mechanistically linked.

46. DETERMINING THE BASIC BIOLOGY OF AN INSECT SPECIFIC FLAVIVIRUS IN AEDES AEGYPTI

Anna C. Dell - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Heather G. Coatsworth** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Insect-specific viruses (ISVs) have been a topic of research over recent years due to their significance and implications in arbovirus research. ISVs belong to a wide range of viral families and are known to only infect their arthropod hosts, as these ISVs are unable to enter and/or replicate in vertebrate cells. Some studies have shown that ISV infection in arthropods may play a role in the insect's competence for other viruses. As such, increasing our knowledge about the basic biology of these ISVs' will be crucial for understanding infection dynamics for mosquito borne viruses such as dengue and Zika. Current ISV dogma suggests that insect-specific viruses are vertically transmitted from mother to offspring, while other routes such as venereal and horizontal transmission play a minor, potentially negligible role in promoting the maintenance of ISVs in populations of medically significant culicids. We hypothesize that a representative ISV, cell fusing agent virus (CFAV) infection in Aedes aegypti will have high vertical transmission rates, and low venereal and environmental acquisition rates. To test if CFAV can be environmentally acquired, we will be assaying larval and pupal source water for CFAV. Completing fully factorial reciprocal mating studies will help us

demonstrate if venereal transmission of CFAV is possible, while assaying eggs will determine if vertical transmission can occur. All individuals studied will be confirmed as CFAV positive or negative using reverse transcription quantitative PCR (RT-qPCR). We will also test CFAV infection levels inside various mosquito tissues through dissections on naturally and inoculated CFAV infected individuals. RT-qPCR will be used to compare titers within and across several Aedes aegypti tissues. All studies will assay if CFAV infection leads to changes in measurable fitness outcomes (longevity, mating success and oviposition). Overall, this study hopes to pin down the transmission route of an ISV and explore the fitness effects that one of these ISVs can have on its host. This research could help contribute to our current and future vector control efforts in our fight against mosquito borne arboviruses.

47. DEVELOPMENT OF A LAB TO GENERATE PRNT50 OUTCOMES TO EXPOSE THE ANTIGENIC SIMILARITIES OF DENGUE 1-4 STRAINS

Luke Trimmer-Smith - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Brooke Borgert** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Dengue is an important human disease-causing arbovirus with four distinct serotypes (DENV1-4) that cocirculate globally in tropical countries. At the Emerging Pathogens Institute (EPI), the Cummings Lab aims to elucidate the antigenic similarity of strains within and between the DENV serotypes. We will be using an optimized Plaque Reduction Neutralization Test (PRNT50) protocol that employs C6/36 (Aedes albopictus) cells, a rich and diverse suite of DENV viruses, and human sera collected in Thailand to expose the antigenic characteristics of each strain tested. With antigenic cartography, the PRNT50 results will be used to produce antigenic maps - providing a way to visualize the antigenic relationships across time and space.

48. DISCOVERY OF THREE NEW ORBIVIRUS SPECIES IN FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) THAT DIED OF HEMORRHAGIC DISEASE IN THE UNITED STATES

John Lednicky - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Mohammad Shamim Ahasan** - College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - College of Veterinary Medicine, University of Florida; **Juan M. Campos Krauer** - College of Veterinary Medicine, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Olivia Goodfriend** - Department of Wildlife Ecology and Conservation, University of Florida; **Caroline Stephenson** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Remi N. Charrel** - Aix Marseille Universite, France; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, University of Florida; **Thomas Waltzek** - College of Veterinary Medicine, University of Florida,

We report the discovery of three novel Orbivirus species found in six dead farmed white-tailed deer in the United States. Phylogenetic analyses indicate that the new orbiviruses are genetically closely related to the Guangxi, Mobuck, Peruvian horse sickness, and Yunnan orbiviruses, which are thought to be solely borne by mosquitos. The novel viruses were found as co-infecting agents along with a known cervid pathogen, epizootic hemorrhagic disease virus-2 (EHDV-2), in four of the dead deer. The occurrence of mixed infections raises questions as to whether the new viruses are primary pathogens or secondary pathogens that exacerbate EHDV-2 infections. Moreover, EHDV-2 is known to be a Culicoides-borne virus, raising additional questions as to whether Culicoides species can also serve as vectors for the novel orbiviruses, if mosquitoes can vector EHDV-2, or whether the deer were infected through separate bites by the insects. Our findings expand knowledge of the possible viral pathogens of deer in the United States. Moreover, due to the close genetic relatedness of the three new orbiviruses to viruses that are primary pathogens of cattle and horses, our findings also underscore a crucial need for additional research on the potential role of the three new orbiviruses as pathogens of other animals.

49. EFFECTS OF TRANSFLUTHRIN EXPOSURES ON MALE AND FEMALE AEDES ALBOPICTUS (SKUSE)

Sean A. McKay - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

The Asian tiger mosquito, Aedes albopictus (Skuse), has become the most invasive mosquito in the world. Spatial repellents are being considered as vector control tools in addition to conventional methods to kill and repel these potential vectors of Zika virus, dog heartworm, and other pathogens. Spatial repellents have demonstrated effectiveness in limiting human-vector contact yet their relationship to the interruption of mosquito pathogen transmission cycles requires further study. This study builds on previous research that determined lethal concentration values for the vapor-active pyrethroid transfluthrin on Ae. albopictus females. In this study, we examined the mortality responses of mosquitoes of differing sex and age classes to low-level concentrations of transfluthrin. Male and female mosquitoes in groups of 2–4 days and 5–10 days old, respectively were exposed separately to transfluthrin vapors, and mortality data was recorded at 2, 4, and 24 hours. Mortality differences in males and females and in age groups will be presented. Understanding mosquito responses following pyrethroid spatial repellent exposure adds data critical to their future use in integrated vector management.

50. EVALUATING EFFECTIVENESS OF VECTOR CONTROL INTERVENTIONS TO ELIMINATE MALARIA FROM NEPAL

Shreejana Bhattarai - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Sadie Ryan** - Department of Geography, College of Liberal Arts and Sciences, University of Florida

Nepal is one of the malaria endemic countries of the world. Malaria was the major cause of morbidity and mortality in Nepal during early 20th century, with nearly half of the population suffering from the disease, and a mortality rate of 10-15%. In the early 1950s, the malaria control program using Indoor Residual Spraying (IRS) with DDT (Dichloro-diphenyl-trichloro-ethane) was initiated, and malaria incidence was significantly reduced. In 1958, Nepal launched the "Malaria Eradication Program", using substantial international aid support, and the number of malaria cases in Nepal dropped to 2,500 by 1970. However, the eradication program then faced financial and technical problems which resulted in its failure; as a result, malaria cases rebounded. Following a renewed global interest in malaria during the 1990s, Nepal began to receive support from the Global Fund in 2004, of which one component was vector control intervention using Long Lasting Insecticidal Nets (LLINs). Malaria transmission once again decreased significantly in Nepal thereafter. At present, Nepal is again in the pre-elimination phase and aims to eliminate malaria by 2026. However, to ensure the success of the current program, it is important to analyze the accomplishments so far, understand what led to previous failure, and identify the challenges ahead. So, the objective of my doctoral research is to evaluate the effectiveness of recent vector control interventions to eliminate malaria from the country. This poster describes the framing of my dissertation work.

51. FRANCISELLA EFFECTORS AT THE INTERFACE OF THE HOST-PATHOGEN RELATIONSHIP

Aria Eshraghi - College of Veterinary Medicine, Department of Infectious Diseases & Immunology, University of Florida

Francisella are host-adapted intracellular bacterial pathogens that cause a deadly systemic disease called tularemia. The species that causes mammalian tularemia, Francisella tularensis, is transmitted via several routes, including aerosols and arthropod vectors, and exposure to fewer than 10 viable bacteria results in upwards of 60% mortality. Although Francisella share a high level of nucleotide identity across the genus, their tropism is diverse, ranging from amoeba to humans. A signature feature of all Francisella genomes is the presence of a gene cluster referred to as the Francisella Pathogenicity Island (FPI) that encodes a type VI secretion system (T6SS). The T6SS is critical for intracellular growth and virulence; however, the mechanism by which it acts is largely unknown. We previously utilized an unbiased proteome-wide approach to identify eight proteins that are secreted by this apparatus in F. tularensis, three of which are encoded outside of the FPI. While deletion of the FPI-encoded effectors results in attenuated intramacrophage growth, concomitant deletion of the effectors encoded within and outside of the FPI abrogates proliferation, similar to a T6SS apparatus mutant. Although the T6SS apparatus is highly conserved among Francisella, homologs of the effectors we identified in F. tularensis are largely absent in symbionts of arthropods or pathogens of fish, suggesting that T6SS effectors may drive host tropism. Characterization of the Francisella T6SS and its effectors will elucidate fundamental mechanisms of the host-pathogen relationship and enable research on countermeasures to infection. Currently, my research program is comprised of three focus areas: 1) Characterization of the activities of mammalian-targeting T6SS effectors, 2) Elucidation of bacterial mechanisms that govern host tropism, and 3) Development of countermeasures to the Francisella T6SS.

52. GOING VIRAL: DOES INSECT-SPECIFIC VIRUS INFECTION AFFECT HUMAN-FLAVIVIRUS TRANSMISSION IN AEDES AEGYPTI?

Heather G. Coatsworth - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Anna C. Dell** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Dengue viruses infect ~400 million people annually and are transmitted principally by Aedes aegypti. Ae. aegypti competency for dengue fluctuates based on the genetic background of the mosquito as well as the virus. It is not well known, however, how indirect factors such as the metagenome affect vector competence. We are interested in knowing if previous and current Ae. aegypti insect-specific virus (ISV) infection affects dengue competency, as negative fitness effects are induced by ISVs closely related to dengue (i.e. cell-fusing agent virus (CFAV)). Previous studies in adult Ae. aegypti investigating CFAV and dengue co-infection led to lower dengue titers. We hypothesize that full CFAV-adaptation through multi-generational CFAV selection in Ae. aegypti will result in reduced dengue competency in comparison to non-adapted CFAV naïve lines. To test this hypothesis, we propose to assess dengue vector competence across three different lines of Ae. aegypti: i) a naturally infected CFAV-adapted line, ii) an experimentally inoculated CFAV-adapted line, and iii) a CFAV-naïve line. CFAV positive offspring will be selected for in treatment groups, while a random offspring selection process will be used for the control treatment. Vector competence will be assessed using plaque assays to determine dengue titer after infecting F10 females from all three experimental lines. This experimental design will allow us to investigate the effect CFAV adaptation has on Ae. aegypti vector competence towards dengue, as well as CFAV infection itself. Overall this research will help us piece together the larger puzzle of dengue vector competence in Aedes aegypti, and could help us develop a predictive flavivirus transmission model based on mosquito exposure and adaptation to insect-specific viruses.

53. IS IT BEST ON THE NEST: EFFECTS OF AVIAN LIFE-HISTORY ON HAEMOSPORIDIAN PARASITISM

Claudia Ganser - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Ara Monadjem** - University of Eswatini; **Robert McCleery** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Infectious diseases exhibit variation in prevalence and pathology among host species. Species may differ in the prevalence of infection due to varying exposure and susceptibility to disease agents throughout their lifetime, which may be attributable to underlying differences in their phenology, physiology and behavior. A recently growing body of literature has focused on the utility of host life-history traits to explain interspecific variation in host-parasite associations. This study utilized the diverse avian and haemsporidian assemblage of Eswatini's Lowveld to evaluate the link between haemoporidia (Plasmodium, Haemoproteus, Leucocytozoon) prevalence and avian life-history traits such as body size, mating system, brood care and nest structure. We found that variation in infection prevalence with haemosporidian parasites was consistent with previously hypothesized associations between life-history traits and transmission rates. Host traits including brood care, nest height, nest type and body size were significantly associated with infection of malarial parasites and other haemosporidia. Brood care was the single most important predictor of infection with haemosporidia in general. The influence of other host traits was less consistent suggesting that differences in the vectors' ecology and host-seeking behavior produce variable patterns of parasitism among haemosporidia genera.

54. KNOWLEDGE, ATTITUDES, AND PRACTICES RELATED TO MALARIA TRANSMISSION BLOCKING VACCINE ACCEPTABILITY IN BO, SIERRA LEONE

Kaci McCoy - CDC Southeastern Center of Excellence in Vector Borne Diseases; **Caroline T. Weldon** - CDC Southeastern Center of Excellence in Vector Borne Diseases; **Rashid Ansumana** - Mercy Hospital Research Laboratory and School of Community Health Sciences, Njala University; **Joseph M. Lamin** - Mercy Hospital Research Laboratory; **David E. Stenger** - Center for Bio/Molecular Science and Engineering, Naval Research Laboratory; **Kevin Bardosh -** Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington; **Kathryn H. Jacobsen** - Department of Global and Community Health, College of Health and Human Services, George Mason University; **Rhoel Dinglasan** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Malaria elimination remains a challenge, with approximately 450,000 deaths occurring each year, primarily in children under the age of five in sub-Saharan Africa. A malaria transmission-blocking vaccine (TBV) targeting Anopheles alanyl aminopeptidase N-1 (AnAPN1), a mosquito midgut enzyme previously shown to be essential for malaria parasite transmission, could help break the cycle of malaria transmission at the community level if coupled with existing interventions. While malaria TBVs are supported in the scientific and regulatory fields, and anecdotally supported by potential users in endemic regions, to date, no formal assessments of TBV acceptability in Sub-Saharan Africa have been conducted. We hypothesized that TBVs, and the AnAPN1 TBV in particular, would be acceptable in relatively malaria intervention-naïve communities and that increased knowledge of (i) malaria transmission, (ii) individual roles associated with transmission, and (iii) how the TBV works by acting at the community level would lead to greater malaria TBV acceptability. To assess this hypothesis, we conducted a mixed-methods study on the knowledge, attitudes, and practices related to the acceptability of a malaria TBV during July 2019 in Bo, Sierra Leone. Participants in the quantitative survey (n = 615) were randomly selected by geographic coordinate within the city limits of Bo. Participants in the six focus groups (each with 7 – 15 participants) and 20 individual

interviews were purposively selected to represent community members with small children and key community health figures, respectively. The survey and discussions focused on views surrounding malaria (e.g. frequency, severity, transmission, population at risk, etc.) as well as vaccines (e.g. willingness receive different vaccine types, previous vaccinations, etc.). Participants were taught how a malaria TBV works to control malaria at the community level before discussing malaria TBV-related questions. Of those surveyed, we found that 99% were willing to vaccinate their child with a malaria TBV and 96% were willing to vaccinate themselves. The majority of those surveyed already participated in malaria prevention and received vaccinations for at least one pathogen, as did their children, representing enablers to malaria TBV acceptability. Cost was a major barrier to vaccine acceptability, with only 56% of those surveyed willing to pay for a malaria TBV for themselves or their child. Knowledge about malaria transmission could also play a role in acceptability, as only 70% of those interviewed identified mosquito bites as the main route of malaria parasite transmission; other commonly identified transmission modes included specific foods or an unclean environment. Ultimately, most surveyed parents and community members in Bo recognized the role they could play in malaria elimination and were willing to receive a malaria TBV such as the AnAPN1 TBV to protect their community. Addressing commonly identified barriers would likely further community malaria TBV acceptability with the end goal of reducing the burden of malaria.

55. LABORATORY AND SEMI-FIELD EVALUATION OF A NEW LARVICIDE AGAINST THREE SPECIES OF MOSQUITOES

Kai Blore - Anastasia Mosquito Control District; **Shougang Zhang** - Nanjing Center for Disease Control and Prevention; **Asaduzzaman Miah** - Anastasia Mosquito Control District; **Whitney Qualls** - Anastasia Mosquito Control District; **Rui-De Xue** - Anastasia Mosquito Control District

Two consecutive studies were conducted to evaluate the efficacy and persistence of the SAFI product as a larvicide against three species of mosquito larvae. SAFI is a commercially available multi-purpose water cleaning agent (active ingredient: 19.8% $CuSO4 \cdot 5H2O$) used primarily to control algae and bacteria. The product was tested via a standard larval bioassay under both lab and semi-field conditions against third instar Aedes aegypti, Culex quinquefasciatus and Anopheles quadrimaculatus larvae. For efficacy, concentrations ranging between 5-100ppm were tested against larvae to determine LC50 values for each species. For persistence, optimal LC50 values of 50, 70, 75 and 90ppm were continuously tested against Ae. aegypti and Cx. quinquefasciatus every seven days for 5 weeks. Study results indicate that concentrations below 5ppm show no observable difference in larval mortality from the control. Of the three species, Cx. quinquefasciatus was most susceptible to the larvicide. The SAFI product was much less toxic to Ae. aegypti (72h LC50lab=28ppm; LC50semi-field=34ppm) than both Cx. quinquefasciatus (72h LC50lab=4ppm; LC50semi-field=14ppm) and An. quadrimaculatus (72h LC50lab=4ppm; LC50semi-field=15ppm) for lab and semi-field conditions respectively. Preliminary study results demonstrate strong persistent effects of the test product with larval mortality at 72h exceeding 80% for both species and both environmental conditions. These studies demonstrate the effectiveness of the SAFI product at low concentrations as a potential larvicide. Additional study is necessary to confirm its effectiveness and further define parameters for real-world application.

56. LANDCOVER COMPOSITION EFFECT ON AMBLYOMMA AMERICANUM TICK SPECIES PRESENCE

Sara Shir - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Claudia Ganser** - Department of Geography, University of Florida; **Greg Glass** - Department of Geography, College of Liberal Arts and Sciences, University of Florida

Ticks feed on blood from different species of vertebrate animals, including humans. During feeding, ticks can transmit micro-organisms to their hosts, as such ticks are important vectors of zoonotic disease agents. Specific tick species tend to transmit one or a few different pathogens. This is significant because the geographic distribution of a tick species limits the geographic range of the pathogen and, consequently, can be an important predictor of disease risk for animals and people. This project is a multiscale investigation of the geographic distribution of the Lonestar tick (Amblyomma americanum). A. americanum are responsible for the transmission of bacteria and viruses such as Ehrlichia, Heartland and Bourbon virus. These ticks have also been linked to red meat allergy and Southern tick-associated rash illness (STARI). Here we evaluated the influence of landcover composition at four spatial scales on the presence of A. americanum on the Florida mainland. The spatial scales ranged from local (50-meter) to landscape level (1000-meter). At all spatial scales, A. americanum presence was most strongly correlated with percent shrubland and forest. No correlation was found with other landcover types (grassland, water or other). The correlation between A. americanum and forest was strongest at the 1000-meter scale (ß=0.042, SE=0.011, $p<0.000$), while the correlation to shrubland was strongest at the 100-meter scale ((ß=0.025, SE=0.007, $p<0.000$). These findings make sense as white-tailed deer, the primary source of blood meal for mature Lonestar ticks, typically reside in woody, forested areas and brush. This finding is significant, as it relates to the potential areas of higher risk of encountering pathogen-carrying ticks.

57. MARK RECAPTURE RELEASE STUDY OF IRRADIATED AEDES AEGYPTI MALES IN ST. AUGUSTINE, FLORIDA

Courtney A Cunningham - Anastasia Mosquito Control District; **Robert Aldridge** - USDA-ARS; **Jedidiah Kline** - USDA-ARS; **Seth C. Britch** - USDA-ARS; **Whitney Qualls** - Anastasia Mosquito Control District; **Chao Chen** - Department of Entomology and Nematology, University of Florida; **Daniel Hahn** - Department of Entomology and Nematology, University of Florida; **Kenneth Linthicum** - USDA-ARS; **Rui-De Xue** - Anastasia Mosquito Control District; **Vindhya Aryaprema** - Anastasia Mosquito Control District

Aedes aegypti are container-breeding mosquitoes found in urban, tropical environments and are the primary vector of arboviruses such as chikungunya, dengue, and Zika. Aedes aegypti populations have been identified in the downtown historic area of St. Augustine, Florida and poses a threat to its fast-growing residential population and its abundant tourism industry. The sterile insect technique (SIT), which targets the mating behavior of mosquitoes for control, has recently been introduced to St. Augustine, Florida for evaluation of its impact on Ae. aegypti populations. To evaluate the potential use of SIT for Ae. aegypti control, a mark-release recapture study was conducted. Irradiated males were provided by the United States Department of Agriculture, Center for Medical and Veterinary Medicine. The males were released at a point location at the evaluation site and monitored using Biogents Sentinel traps placed out twice a week throughout a trapping grid to determine the distance of male dispersal. Additionally, Ae. aegypti adult and egg populations, through ovi-traps, were monitored at both the treatment and control sites to evaluate preliminary releases on abundance. Six releases were conducted. Current findings demonstrate a limited dispersal and no initial reduction in abundance of Ae. aegypti populations in our treatment site compared to the control site.

58. MORE THAN JUST A BITE: ASSESSING PUBLIC KNOWLEDGE OF MOSQUITO-BORNE ILLNESSES

Angela B. Lindsey - College of Agricultural and Life Sciences, University of Florida; **Sandra Anderson** - College of Agricultural and Life Sciences, University of Florida; **Ashley McLeod-Morin** - College of Agricultural and Life Sciences, University of Florida; **Lauri Baker** - College of Agricultural and Life Sciences, University of Florida; **Alena Poulin** - College of Agricultural and Life Sciences, University of Florida

Messages surrounding mosquitos and mosquito control can be confusing and hard to understand for all audiences. In addition, communicating the risk of mosquito-borne illnesses can be complex and could potentially cause unwarranted stress and anxiety. Understanding the challenges of communicating about mosquitos and mosquito control efforts, the Center for Public Issues Education conducted 8 focus groups in four Florida cities (Pensacola, Jacksonville, Orlando, and Miami) to collect information on participants' knowledge and perceptions of mosquito borne illnesses. Two focus groups were conducted in each city in April through May of 2019 for a total of 70 participants for a total of eight focus groups. Participants were recruited from surrounding zip codes in a 10 to 15-mile radius. A moderator guide was utilized for each of the focus groups. All groups were recorded and transcribed. Groups were then analyzed using a constant comparison method to develop themes. Themes included little to a moderate amount of knowledge regarding mosquitos with low knowledge on mosquito control. Majority of participants supported mosquito control, and many used their own personal control methods. Participants were aware that mosquitos carried diseases but were not concerned unless there was an imminent risk or outbreak. Knowledge seeking was low unless participants were aware of a potential risk in their area.

59. ORNAMENTAL PLANTS, AS HOSTS OF TOMATO CHLOROTIC SPOT VIRUS AND ITS VECTOR THRIPS (THYSANOPTERA: THRIPIDAE) IN TOMATO

Rafia A. Khan - Department of Entomology and Nematology, Tropical Research and Eduation Center, College of Agricultural and Life Sciences, University of Florida; **Dakshina R. Seal** - Department of Entomology and Nematology, Tropical Research and Eduation Center, College of Agricultural and Life Sciences, University of Florida; **Shouan Zhang** - Department of Plant Pathology, Tropical Research and Eduation Center, College of Agricultural and Life Sciences, University of Florida

Tomato chlorotic spot virus (TCSV) is an emerging tospovirus in South Florida causing 20-30% yield loss each year. Thrips are the effective vectors of TCSV. Ornamental plants in South Florida show high abundance of thrips of different species. Western flower thrips (Frankliniella occidentalis), common blossom thrips (F. schultzei) and melon thrips (Thrips palmi) are the most common thrips species among them. In this study we used seven ornamental plants as treatments in a greenhouse situation with tomato as a main crop to observe their effect on TCSV incidence and TCSV vector thrips abundance in tomatoes. We have found western flower thrips as the dominant thrips in all ornamental plants. Incidence of TCSV infected tomato plants was observed with all ornamental treatments. Tomatoes with purslane showed higher number of TCSV infected plants. Some of these ornamental plants were found as TCSV reservoir through molecular analysis. The above information will be helpful to develop a sustainable management practice against thrips and thrips transmitted tospovirus problem.

60. ORTHOBUNYAVIRUSES IN THE CARIBBEAN: MELAO AND OROPOUCHE VIRUS INFECTIONS IN SCHOOL CHILDREN IN HAITI

Maha Elbadry - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Ricardo Durães-Carvalho** - Oswaldo Cruz Foundation; **Gabriela Blohm** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Caroline Stephenson** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Sarah White** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Valery Madsen beau de Rochars** - Department of Health Services Research, Management and Policy, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **J. Glenn Morris Jr.** - Emerging Pathogens Institute, College of Medicine, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Medicine, University of Florida,

We report the identification of two orthobunyaviruses, Melao virus (MELV) and Oropouche virus (OROV), in plasma specimens from Haitian children with acute febrile illness who presented during outbreaks caused by alpha- and flaviviruses. MELV, previously thought to be non-pathogenic for humans, was isolated in cell culture from the plasma of five case patients and was phylogenetically related to the only available MELV isolate from Trinidad and Tobago. OROV was detected in the plasma of an additional child, using an unbiased sequencing approach, with phylogenetic inference suggesting a close relationship with strains from Brazil. Abdominal pain was reported by four of four case patients with MELV infections, with lymphadenopathy noted in only two cases. Our findings document the occurrence and spread of these orthobunyaviruses within the Caribbean region and highlight the critical

importance of surveillance (with unbiased sequencing approaches) to identify outbreaks caused by these and other emerging viruses.

61. PLANT ALKALOIDS SYNERGIZE THE MOSQUITOCIDAL EFFECT OF NATURAL PYRETHRINS VIA COMBINED ACTION ON THE NEURONAL VOLTAGE-SENSITIVE SODIUM CHANNEL

Edmund Norris - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

With insecticide-resistant mosquito populations becoming an ever growing concern, new vector control technologies are needed. With the lack of new chemical classes of insecticides to control mosquito populations, the development of novel synergists may improve the performance of available insecticides. We explored the potential of two sodium channel activators, veratrine and aconitine, as both insecticides and synergists of natural pyrethrins on Aedes aegypti adults and larvae. Aconitine was more toxic than veratrine, with an LD50 of 165 ng/mg compared to 300 ng/mg on the pyrethroid-susceptible Orlando strain, but only aconitine showed significant resistance in the pyrethroid-resistant Puerto Rico strain (RR = 14.5). When applied in mixtures with piperonyl butoxide (PBO) and natural pyrethrins, large synergism values were obtained. Aconitine + PBO synergized natural pyrethrins 21.8-fold, whereas veratrine + PBO synergized natural pyrethrins 5.3-fold on the Orlando strain. This synergism was greater than the synergism produced by PBO alone, suggesting another mechanism was present besides that of reduced metabolism. Less synergism of natural pyrethrins was observed on the resistant Puerto Rico strain with acontine + PBO, which synergized natural pyrethrins only 4.1-fold, but veratrine + PBO synergized natural pyrethrins 9.5-fold. These compounds were then applied to the larval mosquito central nervous system (CNS) to assess their potency and synergistic potential directly. When alkaloids were applied alone on both strains, aconitine was less active on the pyrethroid-resistant strain (block at 100 μM) than on the pyrethroid-susceptible strain (block at 10 μM). However, the opposite was true for veratrine, as the pyrethroid-resistant CNS was at least three times more sensitive to this compound. The nerve

blocking effect of NP was synergized significantly by both compounds on the pyrethroid-susceptible strain (approximately 10-fold synergism); however, only veratrine synergized NP block on the pyrethroid-resistant strain (10-fold synergism). These results demonstrate that pyrethroid-resistance in the Puerto Rico strain also produces resistance to aconitine, presumably by altering aconitine binding at the sodium channel. The lack of sensitivity of this strain to aconitine may also explain the lower levels of NP synergism observed in vivo. These results highlight the potential of site II sodium channel activators to synergize natural pyrethroids and may represent future additives to insecticide formulations.

62. POTENT RESISTANCE BREAKING ACTIVITY OF NEW REPELLENT AND INSECTICIDAL ARYL AMIDES

Gary M. Richoux - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Liu Yang** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Edmund Norris** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Quentin Coquerel** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Fabien Demares** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Shiyao Jiang** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Maia Tsikolia** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Kenneth Linthicum** - USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida

Mosquito vectored diseases such as malaria, dengue, and chikungunya continue to be a source of constant concern as millions of people are newly infected alongside the hundreds of thousands who inevitably succumb every year. These debilitating infections are transmitted to humans through the bites of host insects. To-date, one of the most successfully employed methods for preventing the propagation of these diseases has been through the utilization of repellents and insecticides as a means of repelling and killing mosquitoes, intercepting the disease prior to their transmission. For the greater part of a century N,N-diethyl-meta-

toluamide (DEET) has been widely considered the “Gold Standard” of mosquito repellency. More recently, highly potent pyrethroid active ingredients, such as transfluthrin and metofluthrin, have found widespread effective commercial use as repellents and insecticides both indoors and outdoors. While these compounds have seen great successes over the years, the prominent emergence of resistance and lack of total bite prevention highlights the need for new and more potent agents. To this end, we have investigated new potential resistance breaking chemistries, and identified a potent and promising new lead structure consisting of an aryl amide skeleton that exhibited little to no observed resistance and whose facile structural modification allowed for in-depth exploration into activity optimization. Screening the vapor-phase activity of these aryl amides against Aedes aegypti was performed using a horizontal spatial glass tube assay, monitoring both knockdown and mortality. Topical applications to anesthetized mosquitoes was used to screen for contact insecticidal activity. Of the 147 aryl amide analogs synthesized and screened for spatial activity, 25 were found to exhibit greater repellency than DEET. Of these 25 compounds, four had repellencies comparable to transfluthrin and metofluthrin against OR. In most cases, no resistance was observed when the activity against the susceptible Orlando (OR) strain was compared to the pyrethroid resistant Puerto Rico (PR) strain. One derivative had an LC50 three times lower than metofluthrin and within two-fold vapor toxic as transfluthrin. Our investigation into topically applied insecticides, likewise, has provided us with promising data and lead molecules. While our derivatives have not yet reached the lethal efficacy of our target benchmark, propoxur, the most active compounds are within two-fold as toxic to susceptible strains, and against resistant strains our derivatives are 20 to 67 times more potent. Thus, these new chemistries represent excellent candidates for commercial products.

63. PREDICTION OF MICROCLIMATES USING MACHINE LEARNING

Rachel Sippy - Department of Geography, Emerging Pathogens Institute, University of Florida; **Ronald Gangnon** - University of Wisconsin-Madison; **David Gaus** - Salud y Desarrollo Andino; **Diego Herrera** - Salud y Desarrollo Andino; **Jorge Osorio** - University of Wisconsin-Madison; **Jonathan Patz -** University of Wisconsin-Madison

Microclimates are an important component of ecosystems and can impact human health through impacts on vector habitats. Aedes aegypti, the vector of dengue, chikungunya and Zika, is a highly localized species and its abundance is impacted by microclimate conditions. Our understanding microclimate stability is limited; there have been no attempts to predict microclimates. HOBO Temperature/Relative Humidity Data Loggers were deployed in 4 sites per month for 1—24 days each month from September 2016 to August 2017 in a small community in rural Ecuador. Data were summarized for each 24-hour period. We assessed the variability of these summary microclimate measures across time and urban environments. We combined remotely-sensed and climate station data with urbanicity, elevation, and spatial components to predict summary microclimate measures across the entire community using machine learning. Machine learning algorithms were compared and best models were chosen based on predictive ability (highest root mean squared error (RMSE)) for a validation dataset. We collected 287 log-days of data. Some microclimate measures were temporally stable, urban sites had warmer temperature measures and rural sites had higher relative humidity measures. We found that random forest algorithms best predicted many microclimate measures (temperature mean, median, minimum and relative humidity mean, median, RMSE: 0.61—0.65). Generalized boosting models fit temperature and relative humidity variance as well as minimum and maximum relative humidity, with good prediction (RMSE: 0.61—0.72). The best model for maximum temperature was a support vector machine, with moderate accuracy (RMSE: 0.53). Our study was limited by a small sample size over time and space, and limited availability of prediction variables. Machine learning is a promising option for prediction of microclimates, though additional research with large datasets should be conducted for validation of model predictions.

64. RAPID DIAGNOSTIC TEST AIMS TO AID IN THE DIAGNOSIS OF CLINICAL AND SUBCLINICAL PATIENTS LEADING TO A DECREASE IN MALARIA TRANSMISSION

Alexandra Leyte-Vidal - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Timothy Hamerly** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Borja López-Gutiérrez** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Kaci McCoy** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

The evolution of rapid diagnostic tests (RDTs) has increased the accessibility for identification of disease in subjects with limited access to health care due to their ease of use, lack of or limited instrumentation, fast read out, and their inexpensive, robust nature. Malaria transmission in an asymptomatic human reservoir population remains a major enabling factor in the perpetuation of this disease in sub-Saharan Africa. A main barrier to malaria elimination is that a large portion of transmission occurs in subjects with low parasitemia that is undetectable when using the current commercially available blood-based RDTs. We hypothesized that a non-invasive approach with greater acceptability in the population with detection sensitivities approaching that of molecular assays will result in earlier diagnosis and treatment. We have engineered a non-invasive RDT that uses saliva as a sample matrix to detect a parasite-derived novel protein that exists in sexual gametocyte stages and will be able to detect low levels of parasitemia prior to the onset of disease. We collected matching saliva and blood samples from approximately 400 subjects from the Democratic Republic of Congo. Patient blood and saliva samples were evaluated via PCR and RDT, respectively, to determine the concordance between the two detection methods . The comparison to PCR analysis gives us a benchmark for sensitivity and efficacy of diagnosis. We can use this knowledge as guidance to further improve this immunoassay to reach a level of implementation. These results show promise to develop a fieldable device that will ultimately aid malaria elimination and eradication.

65. RAPID INDUCTION OF APOPTOSIS IN AEDES AEGYPTI MOSQUITOS AS AN IMMUNE MECHANISM AGAINST DENGUE-2 AND ZIKA VIRUS INFECTION AT THE MIDGUT INVASION BARRIER

Jasmine B. Ayers - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida; **Seokyoung Kang** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Lei Zhou** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida

Dengue or Zika virus transmission between humans via a mosquito requires infection of the mosquito midgut, followed by spread to the salivary glands so virus can be injected into the bloodstream of the next host during feeding. Some strains of Aedes aegypti mosquitoes are refractory to arbovirus infection and never establish a midgut infection. The mechanism of immunity to infection in the midgut remains unclear. We hypothesize that immediately following ingestion of virus, the mosquito attempts to clear infected cells via apoptosis before viral replication can occur and a midgut infection is established. Within 2 hours of ingesting blood containing dengue-2 or Zika virus, there is a significant induction of pro-apoptotic gene transcripts and apoptotic cells in the midgut of refractory lab-adapted Ae. aegypti strains (Orlando (ORL) and Refractory Moyo-In-Dry (MOYO-R)). Acute inhibition of apoptosis by cofeeding an apoptosis inhibitor with viremic blood corresponds with increased viral genome replication at 48 hours and increased whole-body viral titers at 7 days post-infection. This rapid induction of apoptosis (RIA) may aid in resisting viral invasion of the midgut. This RIA response is not observed in the dengue-2 and Zika susceptible Moyo-In-Dry strain (MOYO-S) which originates from the same inbred lab colony as MOYO-R, suggesting that the differential RIA response could be attributed to genetic differences between the strains. Identifying these genetic predictors for susceptibility to viral infection could aid in arbovirus control efforts by predicting the dengue and Zika outbreak potential of local mosquito populations.

66. REGULATION OF ADIPOKINETIC HORMONE SIGNALING IN MALARIA PARASITE SPOROGONY

Vincent Nyasembe - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Timothy Hamerly** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Prachi Khare** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Borja López-Gutiérrez** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Malaria, caused by Plasmodium parasites, kills close to half a million people and infects more than 200 million every year. A critical step in the complex life cycle is sporogony, which occurs in the mosquito stages of parasite development. During this stage, the parasite undergoes multiple cellular divisions and differentiation over 14 days leading to the generation of genetically diverse, human-infective stages called sporozoites. Consequently, the parasite relies on its Anopheles mosquito vector to provide essential nutrients to support its development. Recent evidence suggests that malaria parasites manipulate the metabolic and immune pathways of its mosquito vectors to increase their transmission potential. We hypothesized that Plasmodium falciparum exploits the adipokinetic hormone (AKH) signaling pathway to mobilize lipid reserves necessary for its metabolic needs and sporogonic development. To investigate this, we tested the effect of P. falciparum infection on the expression of AKH pathway genes in Anopheles gambiae. In addition, we injected P. falciparum-infected Anopheles gambiae with synthetic AKH and studied its effect on parasite development and sporozoite production. Our findings show significant up-regulation of genes in the AKH pathway following P. falciparum infection. Perturbation of AKH levels using synthetic AKH impacted significantly on the parasite sporogony. We discuss the implications of these finding for parasite transmission potential and malaria control.

67. SEASONAL COMPARISONS OF ECTOPARASITES OF WILD MESOMAMMALS IN NORTH-CENTRAL FLORIDA

Sarah Maestas - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Lindsey Campbell** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Wildlife are hosts of ectoparasites that may transmit pathogens of medical and veterinary concern to humans and other animals. Wildlife urban adaptation may result in changes in the ecology of wildlife-associated ectoparasites and vector-borne disease. This research compares the seasonal presence and abundance of ectoparasites of mesomammals captured in north-central Florida. Raccoons and opossums were live-trapped for ectoparasite collection across three seasons at a rural, forested research station to examine differences in the presence, abundance, and species composition of ectoparasites and to serve as a baseline for comparison of ectoparasites of urban-collected mesomammals. A total of 14 sylvatic raccoons and 44 sylvatic opossums were sampled. Ticks of three genera and fleas of two species were collected from raccoons and opossums, while lice were collected only from raccoons, and mites were collected only from opossums. Exploratory data analyses and multivariate statistics were used to examine preliminary data. Specifically, the distributions of ticks, mites, fleas, and lice counts per animal were mapped across the study area, and the differences in the ectoparasite species counts per animal by individual host species investigated. Additionally, the variation in the data set at multiple host/parasite levels was quantified using partial redundancy analyses conditioned on latitude and longitude to test the hypotheses that host species, collection location, and collection season explained a significant proportion of the variance in the ectoparasite data set. This presentation focuses on a subset of preliminary results from a two-year project. Ultimately, this research will help to establish base-line data that

will be used to examine associations between urbanization and diversity of wildlife-associated ectoparasites and vector-borne pathogens that may impact humans and other animals.

68. SOCIAL-ECOLOGICAL INFLUENCES ON DENGUE FEVER AND A COMPARISON OF SURVEILLANCE INDICATORS IN MACHALA, ECUADOR

Catherine A. Lippi - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Anna M. Stewart-Ibarra** - InterAmerican Institute for Global Change Research; **Efraín Beltrán Ayala** - Universidad Técnica de Machala; **Sadie Ryan** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Arboviral diseases are a major threat to public health in Machala, Ecuador (pop. ~280,000), where officials contend with high annual dengue fever burden. Vector control is the primary method of controlling dengue, and management decisions are typically triggered by entomological surveillance and reported human cases. We examined household level social-ecological characteristics to identify factors that promote dengue transmission, and to determine if mosquito presence predicts household dengue cases. Household data on mosquito presence, housing conditions, demography, and dengue prevention practices were collected from 2014–2016 in collaboration with the Ecuadorian Ministry of Health; case data were collected for enrolled households from 2014–2015. Household cluster data were assessed for intraclass correlation, and logistic multimodel selection (criterion=AICc) was conducted to identify factors influencing the presence of mosquitoes or dengue cases, respectively. Protective factors for Ae. aegypti presence in the top model (AICc=536.72) included window screening in good repair, cane housing construction, and air conditioning. Risk factors for dengue cases in the top model (AICc=156.12) included cane housing construction, abandoned housing, shaded patios, and air conditioning, while good housing condition was identified as a protective factor. The relationships between household characteristics and mosquito presence were less defined than those for dengue cases, as evidenced by small effect sizes and model instability (k=240.38). These results highlight the importance of choosing appropriate indicators of transmission activity for public health programs. In locations with hyperendemic transmission, like Machala, targeting

vector control based on human surveillance rather than mosquito counts may be a better management strategy.

69. SPATIO-TEMPORAL VARIATION OF AEDES AEGYPTI, AEDES ALBOPICTUS AND AEDES MEDIOVITTATUS IN GRESSIER AND LEOGANE DURING THE OUTBREAK OF ZIKA VIRUS IN HAITI, 2016-2017

Bernard Okech - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **David Pecor** - Walter Reed Biosystematics Unit, MD; **James Dunford** - Uniformed Services University of the Health Sciences, Bethesda, MD

The recent outbreak of Zika virus in the Americas brought attention to the importance of effective, proactive mosquito surveillance operations, especially in under-resourced countries like Haiti. The Republic of Haiti has one of the highest risk classifications of arbovirus transmission in the Caribbean. The country has recently experienced intense Zika virus transmission, with thousands of reported clinical cases. With no approved human vaccines available for Zika virus, mosquito control is the most effective method for reducing the chances of transmission. Aedes aegypti and Ae. albopictus are the major vectors of the Zika virus. However, detailed studies geared at understating vector mosquito abundance are scarce. In this study, we describe the spatial and temporal population abundance of three mosquito species - Aedes aegypti, Aedes albopictus and Aedes mediovitattus in Gressier and Leogane areas that experienced intense Zika virus transmission from 2016 to 2017. Mosquitoes were caught using BG sentinel traps over a 6-month period. The average mosquito density of Ae. aegypti was higher than Ae. albopictus. Species specific variability in mosquito density by location and over time was observed in the study sites. The data obtained in our mosquito surveillance program will be helpful in the design of proactive approaches to prevent the spread of mosquito borne pathogens in in Haiti and neighboring countries

70. SURVEY OF CHEMORECEPTIVE RESPONSES OF DIFFERENT MOSQUITO APPENDAGES

Liu Yang - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Kenneth Linthicum** - USDA; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Mosquitoes are knowns as the vector of pathogens causing many diseases. Currently, the functions of insect chemoreceptors have been mainly studied on antennae (olfactory receptors) and mouthparts (gustatory receptors). However, other chemoreceptive sensilla are also present on other appendages, such as the tarsi and the anterior wing margin, but their specific roles in chemoreception and mosquito behavior remain largely unknown. In this study, electrophysiological analyses in an electroantennogram recording format were performed on antennae, mouthparts, tars1, and wings to a variety of different insect repellent and attractant compounds. The results provide evidence that the tarsi and wings can sense chemicals in a gaseous form, and there were different responses to different odors on different appendages, but consistent with the strongest response to triethylamine. Antennae and mouthparts showed nearly identical responses to the tested compounds, and their rank orders of effectiveness were similar to those of fore- and mid-leg tarsus. Hindleg tarsi only responded to TEA, indicating that the hind legs are not very chemoreceptive. Wing responded to a range of odorants, but with a different rank order and voltage amplitude. Insights into the function of these organs in insect chemoreception will be discussed.

71. SUSCEPTIBILITY OF AEDES AEGYPTI (ORLANDO) TO DENV-2 AND DENV-4

Caroline Stephenson - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Seokyoung Kang** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Rhoel Dinglasan** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Dengue viruses (DENV-1 through -5) are responsible for the largest number of arbovirus infections among humans globally. Aedes aegypti mosquitoes are the primary vector of DENVs. Barriers preventing DENV dissemination may function differently by viral serotype, causing variable intraspecies vector competence to DENVs. Much of the vector competence literature for Ae. aegypti is biased toward a single lab strain of DENV, DENV-2 New Guinea C strain (NGC). There are hundreds of travel-associated DENV cases in Florida each year with a high percentage of infections originating from the Caribbean as well as Central and South America. We hypothesized that a Floridian strain of Ae. aegypti (Orlando, ORL) would have higher competence for a regionally relevant DENV-4 field isolate versus a long-passaged lab strain. Our rationale is that field isolates are a better representation of currently circulating DENVs among human and mosquito populations in a given region. We examined ORL competence for three different strains of DENV: i) a lab DENV-4 strain H241 (1956), ii) a DENV-4 field isolate from a child in Haiti in 2015 (DENV4/Haiti/0075/2015), and iii) DENV-2 NGC (1944). Mosquitoes were fed either naïve blood (no virus) or virus-spiked blood, then collected on day 7 and day 10 post-blood meal with virus titer quantification via plaque assay. The DENV-4 Haiti group had the highest midgut infection rates on both day 7 and day 10, while DENV-4 lab had the lowest infection rates. In the carcasses however, the DENV-2 lab group had the highest infection rates on day 7, while DENV-4 Haiti had the highest infection rates on day 10. The DENV-4 Haiti group had the highest average midgut titer on day 7, while on day 10, both DENV-2 lab and

DENV-4 Haiti had the highest average titers. These data suggest faster virus dissemination for the DENV-2 lab strain than the DENV-4 Haiti isolate. The DENV-4 Haiti strain may replicate well in the midgut but face a stronger barrier to dissemination, i.e. a Midgut Escape Barrier. Finally, DENV-4 lab had the lowest infection rates and titers which may be because this lab strain is no longer circulating and has diverged from currently circulating DENV-4 strains that may be better adapted to infect Ae. aegypti in the Americas. Further research is needed to pinpoint the genetic difference(s) in these DENV strains that changes Ae. aegypti (ORL) vector competence. This work continues to reveal nuances in Ae. aegypti competence by both DENV strain and serotype, suggesting that future competence work should be specific to mosquito populations of interest and use DENV serotypes/strains that are regionally and medically relevant.

72. SYNCHRONIZATION OF URBAN-RURAL INTERACTIONS: THE ROLE OF WORKPLACE DISTRIBUTION IN DENGUE TRANSMISSION

Thomas Hladish - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Kok Ben Toh** - Department of Statistics, Emerging Pathogens Institute, University of Florida; **Dianela Perdomo** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Sanjana Barhava** - Department of Microbiology and Cell Science, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Despite increased efforts to eradicate dengue in recent decades, the disease's global incidence has continued to rise dramatically, along with the number of severe cases reported. Conventionally, the arbovirus has been regarded as an urban disease, with minimal interventions in rural areas. These regions typically have poorer medical and social infrastructure, aggravating the likelihood of under-reporting. Our current agent-based model of Yucatán, Mexico, though very accurate, lacked workplace data for many rural parts of the state, and because workplaces play a key role in transmission, supplementing this data could prove crucial in affecting disease dynamics within the model. It is possible that the actual dengue burden in Yucatan is greater than anticipated with rural towns playing a role in harboring disease. The aim of this project is to

evaluate how realistic the movement of people in rural areas is within the model, and optimize it to the extent possible given available data and statistical methods. Placing workplaces correctly across rural areas has rendered a more accurate model giving us a better representation of real world dynamics and enhancing our understanding of dengue occurrence, particularly as it relates to the synchronization of urban-rural interactions. Ultimately, we are investigating the role of rural transmission because we suspect not enough resources are allocated to addressing disease burden in these areas, which may play a unique role in dengue's persistence.

73. TICKS AND THEIR VECTORED PATHOGENS WITHIN RURAL, SEMI-URBAN AND URBAN ENVIRONMENTS IN ALACHUA COUNTY, FL

Chanakya Bhosale - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Kristen Wilson** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Carrie De Jesus** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Tick borne bacterial agents and their infections are a steadily increasing threat within the southeastern United States. Florida, USA is home to multiple tick species that can vector bacterial pathogens relevant to human health. In Alachua County, within north central Florida, various cases of in-county acquired tick-borne disease cases have been reported; including Rocky Mountain Spotted Fever and Ehrlichiosis. This investigation analyzed tick species for pathogenic bacterial agents around 15 sites of Alachua County. Placing 5 sites per category, each of the 15 sites were distinguished as rural, semi-urban, or urban based on the presence and amount of visual built environment surrounding the sites. 4 species of ticks were collected using a dragging and flagging method at 8/15 sites in Alachua county. Ticks were all found at a higher abundance within the rural environment. Only one species of tick, Ixodes scapularis was found within semi-urban areas, while both Amblyomma americanum and I. scapularis were found in urban areas. DNA was extracted from each

adult, nymph and pools of larvae. Collected DNA was then used for PCR assays to test ticks for bacterial agents within 4 bacterial genera: Anaplasma, Borrelia, Ehrlichia and Rickettsia. Gel electrophoresis was used to detect positive samples and Sanger sequencing was conducted on those samples to identify specific species. Five bacterial species were detected: Rickettsia amblyomattis, Rickettsia parkeri, Rickettsia spp. endosymbiont, Ehrlichia ewingii, and Ehrlichia chaffeensis. Finding infected ticks well within city limits shows that environmental conditions are ideal for inhabitants; including host abundance and microclimate; but can also be indicative of host movements within and between the different environments. Having ticks with bacterial agents and an increasing number of human and host interactions within all three environments, poses a substantial public health risk.

74. UNRAVELLING THE BIOLOGICAL ROLE OF THE FEMALE GAMETOCYTE MARKER PSSP17 IN PLASMODIUM FALCIPARUM

Borja López-Gutiérrez - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Rhoel Dinglasan** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

According to the most recent estimates, more than 200 million cases of malaria occur each year, causing more than 400,000 deaths. Despite the continuous efforts to control this devastating infectious disease, the reduction of these figures has stalled during the last few years and, more worryingly, has increased in some endemic countries. Asymptomatic individuals with submicroscopic infections contribute to malaria transmission through mosquitoes, and thus their detection and treatment should be considered in elimination strategies. Gametocytes, as the only stage that can be transmitted to the mosquito, are an attractive target for such interventions. In this regard, a female gametocyte marker named Plasmodium sexual stage secreted protein 17 (PSSP17, PF3D7_1218800) was previously identified in the saliva from children with submicroscopic infections. This marker was further validated for the detection of gametocyte carriage with the development of a non-invasive saliva-based lateral flow immunoassay (LFIA) rapid test. Lessons learned from the current generation of malaria rapid diagnostic tests (RDTs) underpin the importance of targeting essential proteins to avoid the emergence of

parasite variants that yield false negative results. However, PSSP17 has not been genetically validated as essential for Plasmodium falciparum survival or transmission. We hypothesize that (i) PSSP17 is dispensable for asexual development but is required for transmission to the mosquito, and (ii) knocking it out could affect female gametocyte maturation, zygote formation and subsequent transmission to mosquitoes. In the present study, we aim to generate a rapamycin inducible PSSP17 knock-out mutant using the CRISPR/Cas9 technology in P. falciparum. Unlike other conventional gene disruption strategies, this approach can unequivocally confirm the essentiality of genes that are not dispensable for the blood stages of the parasite. The characterization of the aforementioned inducible knockout will allow us to dissect the biological role of PSSP17 throughout the complete parasite cycle.

75. USING BAYESIAN MODEL TO PREDICT CAUSES OF CHILDHOOD FEBRILE ILLNESS IN GHANA WITH SYMPTOMS, DEMOGRAPHIC AND HEMATOLOGICAL VARIABLES

Kok Ben Toh - Department of Natural Resources and Environment, College of Agricultural and Life Sciences, University of Florida; **Denis Valle** - Department of Forest Resources and Conservation, College of Agricultural and Life Sciences, University of Florida

Febrile illness is one of the most common symptoms in pediatric illness. While malaria was often the main cause for febrile illness in Sub-Saharan Africa in the past, steady decline in malaria transmission in Africa over the past decades results in larger proportion of fever episodes today to have different etiologies. Lack of accurate diagnostic kits, especially in the remote setting, and the prioritization of malaria has driven up misdiagnoses. Where resources are limited, predictive models and algorithms that rely on predictors that are easily obtained from patients (e.g. demographic characteristics, geographical settings and clinical symptoms) may be used to supplement the existing management framework of childhood febrile illness. Several considerations are important when creating a predictive model based on clinical research data. First, a predictive model needs to overcome the problem of data missingness, which is common in epidemiological and clinical research involving large number of variables. Second, an ideal predictive model would need to be straight-forward to use and easily interpretable by

health practitioners. This challenge can be addressed by incorporating the developed predictive model into a decision support tool, but this solution requires that the predictive model not only generates accurate predictions but also is fast in generating predictions. Here we present a Bayesian model that models the probability of positive diagnostic test outcome using clinical symptoms, demographic and/or hematological variables. The predictors can be a mixture of binary or continuous variables. We use a fully Bayesian approach to impute the missing covariates and predict the outcome simultaneously. We use Bayesian Model Averaging approach to improve prediction accuracy and to conduct variable selection. We then apply the model onto the data from the Acute Febrile Illness study in Ghana, which motivates this study. We compare the model's performance with some common approaches, such as naive Bayes, logistic regression and random forest with or without multiple imputation with chained equations. Finally to demonstrate how this model can be used as part of a decision support tool, we create a prototype tool using the shiny package in R.

76. UV4B AND INTERFERON-ALPHA SUPPRESS DENGUE VIRUS IN RELEVANT HUMAN CELL LINES

Evelyn Franco - Department of Pharmaceutics, College of Pharmacy, University of Florida; **Ashley Brown** - Department of Medicine, College of Medicine, University of Florida

Dengue virus (DENV) is an RNA virus associated with clinical manifestations ranging in severity from self-limiting dengue fever to the more severe and potentially life threatening conditions, dengue hemorrhagic fever and dengue shock syndrome. There are no licensed antivirals against DENV thus, we aimed to evaluate the antiviral potential of four broad spectrum antivirals against DENV in human cells associated with infection. HUH-7 (liver), SK-N-MC (neuronal), and HFF-1 (skin) cells were infected with DENV and exposed to increasing concentrations of either UV4B, interferon-alpha (IFN), favipiravir (FAV) or sofosbuvir (SOF). Viral supernatant was sampled daily and viral burden was quantified by plaque assay on Vero cells. UV4B caused substantial viral suppression in HUH-7, SK-N-MC, and HFF-1 cells, yielding EC50 values of 23.75, 38.77, and 37.38 μM, respectively. Clinically achievable IFN concentrations caused marked declines in peak viral titers in HUH-7 (EC50=102.7 IU/mL), SK-N-MC (EC50=86.59 IU/mL), and HFF-1 (EC50=163.1 IU/mL) cells. SOF exhibited considerable antiviral activity in HUH-7 cells (EC50=6.96 μM) but failed to suppress DENV in SK-N-MC and HFF-1 cells. Treatment with 250 μM FAV produced modest declines in viral burden of approximately 1 and 0.6 log10 PFU/mL relative to the control in HUH-7 and SK-N-MC cells, respectively. FAV was not effective against DENV in HFF-1 cells. IFN + UV4B treatment enhanced antiviral activity in HUH-7, SK-N-MC, and HFF-1 cells relative to monotherapy. In conclusion, our results demonstrate the clinical promise of UV4B and IFN against DENV as they effectively suppress viral replication in multiple clinically relevant cell lines when used as monotherapy and as part of combination regimens.

77. VACCINE FIELD TRIAL FOR EPIZOOTIC HEMORRHAGIC DISEASE VIRUS IN FARMED WHITE-TAILED DEER IN FLORIDA

Yasmin Tavares - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Olivia Goodfriend** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Brandon Parker** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **William Wilson** - Department of Agriculture, US Department of Agriculture; **Jurgen A. Richt** - College of Veterinary Medicine, Kansas State University

Epizootic Hemorrhagic Disease Virus (EHDV) impacts deer and livestock operations globally. In this study, we tested mono-, bi-, and tri-valent vaccines for three serotypes of EHDV in farmed white-tailed deer (Odocoileus virginianus) in Florida. We compared serological status and antibody titers from 39 previously unvaccinated white-tailed deer at three time points. Seven animals were 14 weeks old and 32 individuals were 15 months of age. At day 0, we injected the first dose and collected blood samples; at day 14, we injected a booster and collected blood samples; and at day 33, we collected a final blood sample. We found low titer levels on days 0 and 14, with some outliers. By day 33, most animals in the treatment group responded with a high titer of homologous antibody. Animals injected with a placebo did not show a similar response. At day 33, the difference between treatment and placebo group titers and number of animals that were seropositive were significantly different. We found no difference in titer levels for vaccines when they were administered as mono-, bi- or tri-valent forms. During the course of this study 4 animals died of natural EHDV infection, but none of them received a full vaccine dose. Homologous antibody responses of deer and opportunistic challenge with naturally occurring EHDV suggest that this vaccine may stimulate the immune system sufficiently to protect individuals.

78. WHAT CAN MOSQUITO SPIT TELL US ABOUT HUMAN DISEASE EXPOSURE?

Veronique Etienne - Department of Veterinary Medical Sciences, College of Veterinary Medicine, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Maureen Long** - Department of Veterinary Medical Sciences, College of Veterinary Medicine, University of Florida

There are often challenges associated with trapping mosquitos to determine the specific types that humans are exposed to in a given location. This future work aims to examine mosquito salivary protein as a proxy for exposure to mosquito-borne illness in human populations in the US. The project will consist of a systematic literature review. The information from the review will be used to develop ELISA(s) to test for various mosquito salivary proteins. Furthermore, while some work has been done to elucidate proteins that are immunologically relevant, we aim to use whole saliva to verify our ELISA and screen for additional immunogenic antigens. Lastly, we will use the developed ELISA(s) to screen human samples from various US states to examine the extent and type of mosquito exposure and how that correlates to disease prevalence.

79. WHITE-TAILED DEER CONTACT RATES ON A HIGH-FENCED PROPERTY IN NORTHERN FLORIDA

Jeremy P. Orange - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Maria Uribasterra** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

High-fenced white-tailed deer (Odocoileus virginianus) farms are becoming increasingly common with upwards of 8,000 farms in the United States. The burgeoning deer farming industry has positive financial impacts in the rural communities where farms are often located. However, disease transmission is a significant concern on farms, as animals are often stocked at unnaturally high densities. Furthermore, unlike most other agricultural industries, there are few disease-management protocols in place and less knowledge available to combat disease outbreaks on deer farms. Chronic wasting disease and bovine tuberculosis (Mycobacterium bovis) are two diseases of significant importance to the deer farming industry that involve direct transmission as part of their transmission cycle. Additionally, the transmission dynamics of vector-borne viruses such as epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) may be impacted by direct contacts rates between individuals, as vectoring midges may encounter multiple animals if a blood meal is interrupted. In order to reduce disease transmission on high-fenced properties and to develop management plans for diseases with geographic distributions expanding to novel regions, it is important to have a thorough understanding of disease transmission dynamics and animal contact rates. The objective of this study was to determine how often farmed animals come into contact and if there is any spatial clustering of contact locations. In order to answer these questions, we attached GPS collars to animals between June and August over three years (2015, 2016, and 2017), which corresponds with the primary EHDV and BTV transmission seasons. GPS collars were programmed to collect hourly GPS locations. We compared all combinations (dyads) of animals within each year to identify simultaneous GPS fixes in which animals came within 25m of each other. When a contact was extended uninterrupted over subsequent GPS locations then it was considered a single contact event. Across all three years, dyads came into contact an average of 15.76 times, indicating high

potential for direct disease transmission. Roughly half of all contact events and phases lasted for extended periods of two or more GPS fixes. In addition, spatial analysis indicated contact events were clustered around supplemental feeders, so we suggest that disease intervention strategies be targeted to these sites. The findings of this study can be used when developing disease management plans for diseases that impact the deer farming industry.

80. A BSL-2 PNEUMONIA MODEL OF YERSINIA PESTIS STRAIN KIM5 IN BALB/C MICE FOR EVALUATING ANTIMICROBIAL COUNTERMEASURES

Alexandria Kesterson - Department of Medicine, College of Medicine, University of Florida; **John Craig** - Department of Medicine, College of Medicine, University of Florida; **Renaldo Riggins** - Department of Medicine, College of Medicine, University of Florida; **Lara Chuvala** - Department of Medicine, College of Medicine, University of Florida; **George Drusano** - Department of Medicine, College of Medicine, University of Florida; **Henry Heine** - Department of Medicine, College of Medicine, University of Florida

Introduction: Categorized as a Tier 1 select agent, fully virulent Y. pestis experiments are confined to a biosafety level 3 (BSL-3) laboratory and the types of experiments that may be conducted are heavily regulated. The development of resistant isolates for alternative chemotherapeutic determination are generally denied, necessitating surrogate methods for evaluating resistant isolates. Y. pestis strain Kim 5 has previously been described as an exempt strain surrogate for fully virulent Y. pestis by Schifferli et al 2010 when used intranasally in mice that had been treated with iron providing a model that would not be subject to the regulatory constraints of fully virulent strains.

Hypothesis: Based on these findings, our hypothesis is that antibiotic resistant Kim 5 strains (specifically ciprofloxacin) can be utilized in the iron dosed infection model to assay counter- chemotherapeutics.

Methods & Results: We first determined the minimum inhibitory concentration (MIC) of Kim 5 by broth microdilution for ciprofloxacin & ceftazidime, 0.03 µg/ml & 0.06 µg/ml respectively. We then passaged Kim 5 on ciprofloxacin containing plates to select for a resistant isolate. These isolates were targeted genome sequenced in the gyrA and parC regions and the isolate with the highest MIC and most stable mutation was selected. We then determined the LD50 by whole body aerosol infecting mice with varying concentrations of Kim 5 and Kim 5CR-4. LD50s were 5.8 x 104 and 6.2 x 104 CFU/mouse respectively. A 20-LD50 exposure, natural

history study with animals euthanized every 6 hours for up to 60 hours post infection with lung, spleen, and blood sampled for bacterial burden to determine the course of infection as compared to the fully virulent mode. We conducted a ciprofloxacin dose range in both the parent and resistant strain, with 4 mg/kg/day effective against the parent and verses 60 mg/kg/day showing no effectiveness against the resistant strain.

Conclusions: Our overall conclusion from these data are that the model sufficiently functions as a BSL-2 surrogate for fully virulent infection and that we have successfully utilized a resistant isolate within the model.

81. A SMALL MOLECULE INHIBITS THE TYPE III SECRETION SYSTEM IN PSEUDOMONAS AERUGINOSA PAO1 AND PAK

Young-Sik Kim - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida; **Taimour Langaee** - Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida; **Shouguang Jin** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida; **Virneliz-Fernandez Vega** - Scripps Research Molecular Screening Center; **Timothy Spicer** - Scripps Research Molecular Screening Center; **Thomas Bannister -** Scripps Research Molecular Screening Center; **Louis Scampavia** - Scripps Research Molecular Screening Center; **Paul Gulig** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida

The type III secretion system (T3SS) is a highly conserved complicated protein secretion system in many gram-negative bacteria and functions as an injector of bacterial proteins (effectors) into host cells. Although the T3SS machinery is essential for virulence, it is not essential for bacterial growth outside of an animal host. This makes the T3SS an attractive antimicrobial therapeutic target because inhibitors of the T3SS could potentially reduce bacterial virulence without affecting bacterial growth and survival, thereby avoiding development of resistance. We previously showed that deletion of the ampG gene in P. aeruginosa leads to a very low level of AmpC β-lactamase expression and high sensitivity to β-lactam antibiotics, suggesting that AmpG is a potential target for controlling the resistance to β lactam antibiotics. Through high-throughput screening of more than 645K compounds in P. aeruginosa PAO1, we previously identified compounds that decreased ampC-lux expression and increased

sensitivity to ampicillin. There is evidence that the regulator AmpR, which is a part of the AmpG-AmpC regulatory pathway, also affects expression of the T3SS. Therefore, we evaluated one of the AmpG-inhibiting compounds, UF-S-4, for inhibition of secretion of the T3SS effector ExoS by P. aeruginosa. Interestingly, UF-S-4 inhibited expression and secretion of ExoS in wild-type P. aeruginosa PAO1 and PAK with an IC50 value of 0.88 ug/mL without affecting bacterial growth in broth media. Another compound derived from UF S 4, UF S 18, exhibited similar ampC-lux and T3SS inhibitory activity. In contrast, some other AmpG-inhibitory compounds did not exhibit inhibitory effects on the T3SS. UF-S-4 showed no cell toxicity against HeLa cells. The ampG and ampR deletion mutants were not affected for ExoS secretion. These results suggest that UF-S-4 affects the T3SS in a manner distinct from its effects on AmpG. We conclude that compounds UF-S-4 and UF S 18 significantly inhibit the expression and secretion of T3SS effector ExoS in P. aeruginosa PAO1 and PAK. More compounds are being tested for their potential activities against type III secretion system in P. aeruginosa.

82. ALTERATION OF EXOSPORIUM SURFACE OLIGOSACCHARIDES: EVIDENCE OF CONVERGENT PATHO-EVOLUTION IN BACILLUS ANTHRACIS

Michael Norris - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Alexander Kirpich** - Georiga State University; **Andrew Bluhm** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Diansy Zincke** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Jose Miguel Ponciano** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Bacillus anthracis is a spore forming Gram-positive bacterium that causes the severe disease anthrax. The external surface of the exosporium is coated with glycosylated proteins. The sugar additions are capped with

the unique monosaccharide anthrose. West Africa Group (WAG) B. anthracis have mutations that render them anthrose deficient. Through genome sequencing we have identified two different large chromosomal deletions encompassing the anthrose biosynthetic operon of B. anthracis strains from Chile and Poland. In silico analysis identified the anthrax outbreak among European heroin users was caused by an anthrose deficient strain. Anthrose deficient strains are no longer restricted to West Africa so the role of anthrose in physiology and pathogenesis was investigated in B. anthracis Sterne. Loss of anthrose delayed spore germination and enhanced sporulation. Spores without anthrose were phagocytized at higher rates than spores with anthrose. The anthrose mutant had lower LD50 and decreased time to death of wild type and complement B. anthracis Sterne in the A/J mouse model. Following infection, anthrose mutant bacteria were found at higher numbers in the spleen indicating enhanced dissemination as a result of increased phagocytic uptake of spores. Vaccination studies in the A/J mouse model showed the human vaccine protected against high dose challenges of anthrose mutant. This work demonstrates the importance of understanding emerging pathogens and the role mutation can play in the evolution of pathogenesis. Future work using wild type anthrose deficient B. anthracis is anticipated.

83. AN EXOSOME DERIVED VACCINE CANDIDATE FOR NONTYPHOIDAL SALMONELLA

Lisa E. Emerson - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Winnie W. Hui** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Beatta Clapp** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Mark Ou** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carol Hoffman** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Gustavo Maegawa** - Department of Pediatrics, College of Medicine, University of Florida; **David W. Pascual** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Mariola Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Nontyphoidal Salmonella (NTS) has emerged as a public health concern causing an estimated 95.1 million infections and over 50,000 deaths each year. Children under five, the elderly, and immunocompromised people have a higher risk of death associated with infection. NTS is acquired by ingesting food or water contaminated with feces. Once in the body, NTS can hijack host macrophages, which in response secrete exosomes. Exosomes are small extracellular vesicles used by the host immune system for intercellular communication. Exosomes carry cargo, including proteins, RNA, metabolites, and lipids, which are then transmitted to other cells. These secreted exosomes can induce host immune responses. The goal of this project is to characterize further the cargo contained in Salmonella infected macrophage exosomes and develop a potential exosome-derived vaccine strategy against NTS. Previous studies have uncovered several possible vaccine strategies to combat infection including a vaccine containing the SopB virulence factor. Since SopB has been one of the proteins encapsulated within exosomes, in this project, we will further analyze pro-inflammatory exosomes containing SopB through flow cytometry, fluorescent microscopy, and western blots. We expect that these pro-inflammatory exosomes will contain SopB

trafficked therein in a ubiquitin-dependent manner and that Salmonella antigens and will be suitable as a vaccine candidate. We will finally use a murine model to characterize humoral and cell-mediated responses to the SopB-containing exosomes.

84. ANISOTROPIC SILVER NANOPARTICLES : DEVELOPMENT OF AN ANTIMICROBIAL GEL FORMULATION FOR WOUND HEALING IN ANIMAL

Saengrawee Thammawithan - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Rina Patramanon** - Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon kaen, Thailand

The excessive use of antibiotics in both human and veterinary medicine has contributed to the development and rapid spread of drug resistance in bacteria. Silver nanoparticles (AgNPs) have become a tool of choice that may be used to treat these resistant bacteria. It has been demonstrated by several studies that AgNPs had antibacterial and wound healing properties. In this study, we aimed to develop an antimicrobial gel formulation containing anisotropic AgNPs for treating wound infection in animals. We showed that some anisotropic AgNPs (S2) had good antibacterial activity against bacterial pathogens and low cytotoxicity to keratinocytes and fibroblasts in vitro. The MIC and MBC values were in range of 2-32 µg/mL and the cytotoxicity had the IC50 values at 62.02 µg/mL and 66.52 µg/mL against HaCaT and NHDF cells, respectively. The anisotropic AgNPs (S2) was then used as a gel component and tested for antibacterial activity, including long-term protection compared with povidone iodine, a common antiseptic agent. The study has shown that the anisotropic AgNPs can inhibit growth of most tested bacterial pathogens, as well as providing a protection longer than 48 hours, while the povidone iodine inhibits growth of bacteria only in 24 hours. We also evaluated the antimicrobial and the wound healing activity of anisotropic AgNPs gel in BALB/c mice. Interestingly, the anisotropic AgNPs gel was not only cure the infection, but also be able to remove the scar within 21 days better than the povidone iodine. This study has suggested that the anisotropic AgNPs could be used as an alternative antimicrobial agent for

treating bacterial skin infection and wound healing formulation in animals.

85. ASSOCIATIONS BETWEEN COGNATE AND NON-COGNATE ANTIBIOTIC USE WITH ANTIBIOTIC-RESISTANT URINARY TRACT INFECTIONS

Shannan Rich - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Inyoung Jun** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **J. Glenn Morris Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Mattia Prosperi** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida

Introduction: Urinary tract infections (UTIs) are among the most common bacterial infections in the US. These infections frequently recur, and recent data suggest they are becoming resistant to some of the most common antibiotics used to treat them, including Trimethoprim-Sulfamethoxazole (SXT) and Ciprofloxacin (CIP). Electronic medical records (EMR) data, including history of UTIs and antibiotic prescriptions, have been used to predict antibiotic-resistant UTIs in non-US populations. The objective of this study was to assess the relationship between past antibiotic use, distinguishing between drug types [cognate (same drug) vs. non-cognate (different class)], and antibiotic resistant UTIs in a US population.

Methods: We obtained anonymized EMR data for all individuals meeting the following inclusion criteria: 1) aged 18 years or older, 2) diagnosed with a UTI for which an antibiogram test was performed at UF Health between 2011 and 2019. Records included patient demographics, previous relevant diagnoses, past antibiotic prescriptions, and antibiogram results. Boosted multivariable logistic regression models were fitted to assess the relationship between prior antibiotic use and antibiotic-resistant infections while adjusting for confounding factors.

Results: There were 10,340 unique patients who met the study criteria. The population was majority female (76.4%), white (67.7%), and non-Hispanic (96.1%) with a mean age of 60.7 years (STD=19.8 years). The proportion of infections resistant to at least one antibiotic was 63.0%, ranging between 58.8% and 67.1% throughout the study period. The most common uropathogens were Escherichia coli (59.1%), Klebsiella pneumoniae (14.6%), and Enterococcus faecalis (5.5%). In the multivariable analysis, past antibiotic use was significantly associated with resistance to at least one antibiotic (odds ratio = 1.23; 95% confidence interval = 1.10-1.38), after adjusting for known confounders (diabetes, history of UTI, pregnancy, and immune deficiency). Among the top three antibiotics prescribed for UTIs (SXT, Nitrofurantoin [NIT], and CIP), resistance was linked to significantly higher odds of past cognate and non-cognate antibiotic use for SXT and CIP.

Conclusion: Recent antibiotic use, along with comorbid diabetes and a history of UTIs are strongly associated with SXT and CIP resistance – two of the main antibiotics prescribed to treat UTIs. These factors are easily accessible in patients' electronic medical records and can perhaps be used to inform treatment decisions in settings where susceptibility testing is not routine (e.g. outpatient facilities). In the post-antibiotic era, improving the use of existing antibiotics, including CIP and SXT, is essential in the battle against antimicrobial resistance.

Acknowledgements: This work has been supported by the University of Florida's "Creating the Healthiest Generation" Moonshot initiative, supported by the UF Office of the Provost, UF Office of Research, UF Health, UF College of Medicine and UF clinical and Translational Science Institute.

86. AUSTWICKIA CHELONAE IN A WILD GOPHER TORTOISE (GOPHERUS POLYPHEMUS): EVIDENCE OF POSITIVE SELECTION IN THE DIPHTHERIA-LIKE TOXIN GENE

Brittany Liguori - College of Veterinary Medicine, University of Florida; **Robert J. Ossiboff** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Erin Graham** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Nicole Stacy** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Rachel Turner** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Jane Christman** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **April Childress** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Amy Alexander** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Darryl Heard** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **James F.X. Wellehan** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida,

Austwickia chelonae, previously Dermatophilus chelonae, is a filamentous, gram positive Actinobacterium that belongs to the Dermatophilaceae family. It has been associated with fatal granulomatous disease in diverse captive reptile species on three continents. An adult female Gopher tortoise (Gopherus polyphemus) was presented to the University of Florida Zoological Medicine department after being found dehydrated, cachectic and infested with ticks, with two visible masses over the cranioventral neck and right stifle. Complete blood count and serum biochemistry did not reveal any significant abnormalities, however, an elevated erythrocyte sedimentation rate was present. Computed tomography revealed three well defined masses, and cytology of fine needle aspirates of the two visible masses revealed abundant necrotic debris and frequent focal aggregates of cocci and diplococci. Following surgical excision, histology revealed chronic granulomas with intralesional filamentous bacteria. Pan-bacterial 16S rRNA polymerase chain reaction of the excised masses followed by direct sequencing identified Austwickia chelonae. Despite treatment with oxytetracycline and ceftazidime the patient did not improve and

ultimately did not survive. No further masses consistent with Austwickia chelonae were seen on necropsy and it was determined that the tortoise ultimately succumbed to fungal pneumonia. This report reveals the first documented case of Austwickia chelonae in a wild animal outside of captivity. This organism produces a toxin gene similar to diptheria toxin, which has not previously been seen outside the genus Corynebacterium. The toxin and rpoB genes were amplified and sequenced. Selection analysis revealed that the toxin gene is under positive selection, which implies it interacts significantly with the immune system, though the rpoB gene was heavily conserved. Since this is the first documented case of Austwickia chelonae in a wild animal, the environmental reservoir is unknown, and overall, this organism has the potential to have significant impacts on this already declining species, as well as others.

87. BURKHOLDERIA UBONENSIS TETRACYCLINE RESISTANCE: THE INTERPLAY BETWEEN RND AND MFS TRANSPORTER MEDIATED EFFLUX

Nawarat Somprasong - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Jessica R Webb** - Global and Tropical Heath Division, Menzies School of Health Research, Darwin, Northern Territory, Australia; **Carina M. Hall** - The Pathogen and Microbiome Institute, Northern Arizona University; **David M. Wagner** - The Pathogen and Microbiome Institute, Northern Arizona University; **Paul Keim** - The Pathogen and Microbiome Institute, Northern Arizona University; **Herbert P. Schweizer** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida

The antibiotic resistance crisis is one of the biggest challenges of public health in our time. The exchange of genetic material across genera, in term of horizontal gene transfer, contributing to antibiotic resistance of harmful bacteria is dramatically increasing worldwide. Burkholderia ubonensis (Bu) is a non-pathogenic bacterium that frequently is co-isolated from the environment with B. pseudomallei (Bp), the causative agent of melioidosis. Concerns arise as Bp might increase its already significant drug resistance by acquisition of DNA from drug resistant near neighbor species such as Bu. Interestingly, tetracycline (TET) resistance in Bu is common across the species but the underlying mechanism(s) are currently unknown. We used molecular genetic tools to investigate the Bu

TET resistance mechanisms in strain Bp8955, a highly TET (minimal inhibitory concentration [MIC] ≥ 256 µg/ml) and doxycycline (DOX; MIC = 32 µg/ml) soil isolate from Puerto Rico. Random transposon mutagenesis was used to identify mutants with increased TET susceptibility. The screening of approximately 2,000 transposon mutants revealed two TET susceptible mutants. They contained transposon insertions in tetA and amrB, encoding a major facilitator superfamily (MFS) efflux transporter and a membrane transporter protein of the resistance nodulation cell division (RND) AmrAB-OprA efflux pump, respectively. The mutant with a transposon insertion in tetA exhibited TET (MIC = 16 µg/ml) and DOX (MIC = 3 µg/ml) susceptibilities that were 16-fold lower than its parental strain. The amrB mutant showed TET (MIC = 96 µg/ml) and DOX (MIC = 3 µg/ml) susceptibilities that were 3 to 16-fold lower than its parental strain, respectively. Neither mutation affected the minocycline (MIN) susceptibility (MIC = 2 µg/ml) of Bp8955. These results indicate that both TetA and AmrAB-OprA are required for tetracycline resistance in Bu. In addition, whole genome sequence analysis of Bukholderia cepacia complex (Bcc) and Burkholderia pseudomallei complex (Bpc) bacteria revealed that TetA is only present in Bu. Although TetA is a common TET resistance determinant in Gram-negative bacteria Bu is unique because the tetA gene together with is TetR repressor gene tetR is present in the chromosome rather than being contained on a transposon. Taken together, our data confirm that the intrinsic high-level TET resistance of Bu is due to the interplay of a RND efflux pump and a TET MFS transporter.

88. CHLAMYDIA TRACHOMATIS GENES CTL0323-0326 ENCODE A FUNCTIONAL IRON TRANSPORT SYSTEM

Jessica A. Slade - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Daniel Groso** - Emerging Pathogens Institute, University of Florida; **Michelle Qu** - Emerging Pathogens Institute, University of Florida; **Lily McKnight** - Emerging Pathogens Institute, University of Florida; **Anthony T. Maurelli** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Chlamydia trachomatis is an obligate intracellular, Gram-negative pathogen responsible for over 130 million new genital tract infections each year worldwide. For over 20 years it has been known that low iron conditions, achieved by exposure to iron chelators like 2,2'- bipyridyl (BPDL), cause C. trachomatis to leave normal development and enter a state known as persistence. In this state, chlamydiae remain viable, increase in size, but cease dividing. Iron deprivation induced persistence is reversible if cultures are replenished with fresh iron-containing media. These data indicate that C. trachomatis can transport iron, but no iron transport system has been characterized in Chlamydia. Iron acquisition is critical for all bacteria, thus multiple iron transport systems have been identified in Escherichia coli and the facultative intracellular pathogen, Shigella flexneri. Bioinformatic analysis comparing the E. coli and S. flexneri systems to the C. trachomatis L2 genome revealed that the S. flexneri sitABCD iron transport system is homologous to four chlamydial genes, ctl0323-0326. Previous studies have demonstrated that protein Ctl0323, also known as YtgA, has iron binding capabilities in vitro. Based on these data, together with the operon organization and protein homology with SitABCD, we hypothesized that Ctl0323-0326 comprise an iron transporter in Chlamydia. To test this hypothesis, we utilized an iron transporter deficient double mutant strain of S. flexneri, BS934. Transformation of BS934 with the E. coli-C. trachomatis shuttle vector, pREF100 harboring ctl0323-0326 improved growth of BS934 compared to the BS934 empty vector control in liquid culture, even when iron was depleted from the media using BPDL. The intracellular environment has less free iron compared to liquid culture, therefore, we were interested in whether overexpression of ctl0323-0326 would also improve intracellular

invasion and replication of BS934 in tissue culture assays. No difference in invasion of mammalian cells was observed between the wild type strain, BS934 and BS934 complemented with ctl0323-0326. However, overexpression of ctl0323-0326 in BS934 significantly improved the ability of this mutant to form plaques in mammalian cells, which requires the bacteria not only to survive intracellularly, but to replicate and spread cell-to-cell. We conclude that ctl0323-0326 comprise an iron transport system in Chlamydia and that expression of these genes is particularly important for promoting intracellular survival.

89. DIFFERENTIAL GENE EXPRESSION PATTERNS OF LEPTOSPIRA USING A WHOLE BLOOD CULTURE STIMULATION SYSTEM

Dhani Prakoso - College of Veterinary Medicine, University of Florida; **Alejandro Llanes** - Instituto de Investigaciones Científicas y Servicios de Alta Tecnología de Panamá; **Gina Hinton** - College of Veterinary Medicine, University of Florida; **Sreekumari Rajeev** - College of Veterinary Medicine, University of Florida

The role of host innate immune system in Leptospira infection and outcomes such as clearance, asymptomatic colonization versus disease is unknown. The objective of this study was to identify the host and bacteria specific gene expression patterns upon initial encounter with each other in the blood. We hypothesized that,, the exposure of host whole blood with Leptospira strains will result in differential host and Leptospira specific gene expression patterns. We performed exploratory experiments by stimulating bovine whole blood with pathogenic strain L. interrogans serovar Copenhageni and nonpathogenic strain L. biflexa serovar Patoc. We extracted total RNA and generated cDNA libraries for sequencing using the Nanopore MinION platform. Raw read counts normalized by a regularized logarithmic transformation was used as a proxy for gene expression, in order to identify differences between the cultured strain and that exposed to blood for each of the two species. For L. interrogans serovar Copenhageni, 2,868 genes showed evidence of differential expression, of which, 58.09% were putatively upregulated and 41.91% were putatively downregulated. In the case of L. biflexa serovar Patoc, we found evidence of differential expression for 2,126 genes where 52.63% were putatively upregulated and 47.37% were putatively downregulated. Among the genes suspected to be differentially

expressed, 1,170 are common to the Copenhageni and Patoc serovars, thus putatively belonging to the Leptospira core genome. This preliminary analysis suggests that there are in fact differences in gene expression in bacteria exposed to host blood and confirms the feasibility of the suggested approach to study such differences. Due to the relatively low capacity of the Nanopore MinION platform, we could not study the differential expression patterns of the bovine host in these exploratory experiments.

90. DIVERSE B-LACTAM ANTIBIOTIC RESISTANT BACTERIA AND MICROBIAL COMMUNITY IN MILK FROM MASTITIC COWS THAT MAY NOT BE RELATED TO ANTIBIOTIC TREATMENT FAILURE

Zhengxin Ma - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Shinyoung Lee** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Peixin Fan** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Jaehyun Lim** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Klibs N. Galvao** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Corwin Nelson** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Intramammary bacterial infections, the most common cause of mastitis, is the most costly disease in dairy cattle in the US and reason for antibiotic usage. Ceftiofur, a third generation cephalosporin, is generally used to treat such disease, but it has a high failure rate. Though the reason is not known clearly, it is hypothesized that multiple factors are associated with the treatment failure, including antimicrobial resistant bacteria. In this study we analyzed 169 milk samples from cows with mastitis in two independent dairy farms (Farm A and B) in which 19.4% (Farm A) and 14.3% (Farm B) of the antibiotic treated cows were not cured. The prevalence of cephalosporin resistant bacteria (CRB) in milk was 72.0% and 42.1% in Farm A and B, respectively. Nineteen and nine

genera were identified in Farm A and B respectively, with the most abundant genus being Staphylococcus (27.1%; Farm A) and Bacillus (63.5%; Farm B) However, no relationship between treatment efficiency and the CRB prevalence was observed. Furthermore, the metagenomic analysis showed no significant differences in the α- and β-diversities of microbiota in milk samples from cured and non-cured cows, suggesting that antibiotic resistant bacteria were not the key reason for antibiotic treatment failure.

91. ESTIMATING THE LOCAL INFECTIOUS ZONE AND RISK FACTORS FOR ANTHRAX TRANSMISSION IN WEST TEXAS

Jason Blackburn - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Diansy Zincke** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Morgan Walker** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Michael Norris** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

The local infectious zone, or LIZ, has been defined as an area around an animal carcass where another animal can become infected with an environmentally maintained pathogen. This definition was first proposed for anthrax-caused carcasses in Etosha National Park, Namibia and is often related to carcasses in open grasslands where vegetation is low and flies play little role in feeding on carcasses or biting animals while alive. In West Texas, the situation is quite different, where most carcasses are found in densely shaded scrub habitats, blow flies feed on carcasses extensively and contaminate surrounding browse (vegetation eaten by other hosts), and biting flies feed aggressively on hosts. While work in Namibia and Montana has characterized the LIZ and how hosts interact with those LIZs (grazing at or defending territory to graze at), less work has characterized the LIZ in scrub habitat or confirmed the role of biting flies. Here we do both using data from several West Texas outbreaks, and new direct evidence of biting flies feeding on dying and freshly dead animals and containing infectious dose quantities of viable Bacillus

anthracis, the causative agent of anthrax, from the host (confirmed through whole genome sequencing). Evidence from our field investigations confirms that fly emesis and feces near carcasses are loaded with viable B. anthracis. Additionally, we confirmed that leaves of browse species have viable organism for at least several days after animal death, and in all positive leaves tested from the 2019 outbreak, both plasmids are present, suggesting the organism was infectious at the time of collection. We use these results to provide evidence for indirect transmission between hosts during outbreaks through biting flies and to illustrate a 3D LIZ in scrub habitats. Both pieces of information can be used to guide surveillance, decontamination efforts, and to inform wildlife managers or risk to hosts and humans in the West Texas Enzootic Zone.

92. IDENTIFICATION AND QPCR TEST DEVELOPMENT FOR A NOVEL MORTALITY-ASSOCIATED HELICOBACTER SPECIES IN GOPHER TORTOISES

Tasha M. Desiderio - Department of Small Animal Clinical Sciences, College of Agricultural and Life Sciences, University of Florida; **Nicole Stacy** - College of Veterinary Medicine, University of Florida; **Robert J. Ossiboff** - College of Veterinary Medicine, University of Florida; **Marley Iredale** - College of Veterinary Medicine, University of Florida; **Linda L. Archer** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Amy Alexander -** College of Veterinary Medicine, University of Florida; **Darryl Heard** - College of Veterinary Medicine, University of Florida; **Sarah E. Crevasse** - College of Veterinary Medicine, University of Florida; **Kyle A. Donnelly** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Kyle A. Donnelly** - College of Veterinary Medicine, University of Florida,

The genus Helicobacter are spiral shaped bacteria in the phylum Proteobacteria, order Campylobacteriales that have been associated with disease in animals, including reptiles. Three gopher tortoises (Gopherus polyphemus) presented to the University of Florida between 2012 and 2019 with nasal discharge, depression, and weight loss. Cytologic examination of nasal discharge found uniform populations of spiral shaped bacteria. PCR and sequencing of the 16S rRNA gene revealed this

to be a novel Helicobacter species. Genus-specific primers were designed and the gyrA and groEL genes were further amplified by PCR and sequenced. Development and validation of two probe hybridization qPCR assays for sensitive, specific, and quantitative detection was performed. These assays were used to survey nasal wash samples from a collection of 37 gopher tortoises. Assessment of each tortoise's medical records were compared with qPCR results for this bacteria. Specific information, including gender, medication given, nasal discharge score, blood values, and other clinical data were evaluated. Mortality of tortoises was significantly correlated to the loads detected by qPCR for the gyrA and groEL genes. Phylogenetic analysis is underway.

93. IDENTIFICATION OF BACTERIOPHAGES SPECIFIC TO BURKHOLDERIA PSEUDOMALLEI STRAINS

Pacharapong Khrongsee - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Bryn E Tolchinsky** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Aleeza Kessler** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Burkholderia pseudomallei is a Gram-negative bacterium dwelling in soil and water as well as the causative agent of melioidosis in both humans and animals. Melioidosis is endemic in tropical regions, mainly Southeast Asia and Northern Australia. Due to the development of antibiotic resistance, the expense of effective treatment with a specific antibiotic regimen for up to 20 weeks, and the difficulty of decontamination of the infected area, melioidosis is often considered untreatable in animals. Bacteriophage therapy is now being explored as an alternative treatment for this infection. In pursuit of determining the best phage for downstream application, a screening process was designed to identify the receptors, other than the O- antigen receptor, that enable the phage to overcome the global majority serotypes of the bacteria. The phages were selected using two virulence attenuated strains of B. pseudomallei—Bp82 (serotype A) and 576mn (serotype B)—designed for the experiment to represent the two major serotypes. One hundred and forty-five phage

samples isolated from B. pseudomallei sequence type 3 (wildtype) from 24 unique locations in the Songkhla province of Thailand were sent to University of Florida for analysis. Ten out of 145 samples overcame the differences of LPS, resulting in positive plaque formation for both strains. Twenty three bacteriophages were isolated from those 10 samples, then ran through a panel of genetically diverse B. pseudomallei strains and other genetically related Burkholderia species including members of B. pseudomallei complex and B.cepacia complex. Fifteen out of 23 (65%) phages can infect up to 70% of B. pseudomallei strains of both serotypes, while none of these phages can infect other Burkholderia species except atypical B.thailandensis TXDOH and B. mallei ATCC 23344. We also noted that most of these phages did not infect other B. mallei strains. A second panel was conducted to determine receptors for infectivity using mutants for capsular polysaccharide (CPS), O-antigens, or both. This panel revealed that CPS and O-antigens are each essential for phage infectivity. In summary, this method can both select specific phages that landed on multiple receptors specific to B.pseudomallei. The results from this experiment will provide the best phages for downstream applications, including phage engineering and phage therapy.

94. IDENTIFYING THE MECHANISM FOR CHLAMYDIA TRACHOMATIS GENETIC TRANSFORMATION

Louise M. Ball - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Anthony T. Maurelli** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Natural competence is the ability of bacteria to take up extracellular DNA and use the genetic information encoded within it. This process is well studied in a variety of bacteria and several systems for DNA uptake have been characterized. Chlamydia trachomatis is a sexually transmitted Gram negative obligate intracellular bacterial pathogen responsible for 130 million new infections worldwide annually. Chlamydia are unique compared to most bacteria, as they undergo a biphasic “developmental cycle” starting with infectious elementary bodies (EB) which invade susceptible host eukaryotic cells. Once inside the cell, the EB differentiates into its replicative form, the reticulate body (RB) and multiplies to produce more RBs in a protective vacuole called an “inclusion”. As the developmental cycle reaches the end, RBs differentiate back to EBs and the bacteria exit the host cell. During infection, if two bacteria infect the same host cell, their respective inclusions will combine in a process called homotypic fusion. Prior in vitro studies have shown exchange of genetic traits between genotypically different C. trachomatis, producing a chimeric progeny. This suggests that chlamydiae are naturally competent. One mechanism for natural competence is the type 2 secretion system (T2SS) which has been implicated in natural transformation of Campylobacter jejuni and Klebsiella oxytoca. DNA transformation of C. trachomatis in vitro is an inefficient process and is complicated by the biphasic nature of the bacteria- the infectious EB is metabolically inactive, but the potentially competent RB is non-infectious. Here we show that DNA exposed to C. trachomatis bacteria remains in a “DNase protected state”, suggesting that the transformation event occurs before entry into host cells. This study also proposes to investigate the T2SS as a DNA uptake mechanism for C. trachomatis, by overexpressing T2SS components and using qPCR to measure DNA uptake. We also propose to investigate a potential role for RBs during in vitro transformation. This study will answer fundamental questions

surrounding competence and DNA transformation of C. trachomatis in vitro, leading to better understanding of the process. It will also provide ideas for improvement of the in vitro methodology for Chlamydial genetic transformation which remains an inefficient process after its initial publication nearly a decade ago.

95. INSIGHTS ABOUT THE GENETIC DIVERSITY OF PHYTOPLASMAS IN FLORIDA AND THE CENTRAL AMERICA IN BOTH PALM HOSTS AND VECTORS BASED ON 16S HIGH-THROUGH PUT SEQUENCING

Marina S. Ascunce - Department of Plant Pathology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Marie de Gracia Coquerel** - Department of Plant Pathology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Lidia Komondy** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **De-Fen Mou** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Alessandra R. Humphries** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Noemi Soto** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Ericka E. Helmick** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Erica Goss** - Department of Plant Pathology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Edwin A. Barrantes** - Universidad of Costa Rica; **Brian W. Bahder** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida,

Phytoplasmas are plant pathogens that impact a wide variety of agricultural (e.g. coconuts, date, sugar cane) and ornamental crops. These uncultured bacteria are transmitted primarily by phloem-feeding hemipteran insects, including leafhoppers, plant hoppers and psyllids. Among the more serious phytoplasma diseases are the lethal yellowing-like diseases of palms that result in yellowing, wilting and death of palms causing major outbreaks that led to the losses of millions of coconut and other palm species. Since 2006, a phytoplasma disease known as Lethal Bronzing has infected more than 16 palm species in Florida, thus posing a threat to the Florida landscaping and nursery industry, where palms

account for a revenue of $400 million annually. In this initial study, we collected 89 palm samples: 54 from Florida and 35 from Costa Rica, plus 18 hemipteran insects in Florida, which are considered potential vectors of phytoplasmas. DNA extractions were subjected to high throughput amplicon sequencing method using group-specific taxonomic barcode: 16S (V4 region) for bacteria, which allows the description of the total bacterial community. Preliminary analysis of a subset of those samples indicated the presence of phytoplasma in all. Potential endosymbionts in the order Enterobacteriales were detected in the hemipteran insects, which could be characterized for their potential for vector control. We are continuing to sample both palms and vectors in Florida and the Caribbean to assess the diversity of palm-infecting phytoplasmas, their vectors and their whole microbial community.

96. MOLECULAR SURVEILLANCE OF CEFTAZIDIME (CAZ) RESISTANCE IN MELIOIDOSIS

Iris F. Irby - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Mohammad Siddiqur Rahman Khan** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Yukari Fukushima** - Hokkaido University; **Yuta Kinoshita** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Yuki Ouchi** - Hokkaido University; **Yasuhiko Suzuki -** Hokkaido University; **Chie Nakajima** - Hokkaido University; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Melioidosis is caused by Burkholderia pseudomallei, a Gram-negative bacterium that is found in soil and water in most tropical regions but predominantly in Southeast Asia and northern Australia. Treating melioidosis is complicated because the bacterial pathogen is resistant to most antibiotics used in the initial empirical management of sepsis. Ceftazidime (CAZ) is the primary intravenous (IV) drug of choice recommended for treating melioidosis to prevent death from sepsis. CAZ is a third-generation cephalosporin antibiotic with bactericidal activity. CAZ resistance in melioidosis patients has emerged in most Southeast Asian countries in recent years. The primary goal of this project was to

identify genetic and molecular basis of CAZ resistance mechanisms in a large collection of B. pseudomallei collected from a longitudinal cohort study in Thailand. This study has demonstrated that mutations of penA, a class A β-lactamase gene, can potentially cause CAZ resistance. Based on the findings from patients who had treatment failures in Northeast Thailand during 1987-2007, our study has suggested strong associations between the increased minimal inhibitory concentrations of CAZ and multiple amino acid substitution (AAS) mutations in PenA, a specific penA promoter-up mutation -78A, and/or multiplication of penA in most CAZ resistant B. pseudomallei strains. The multiplication of penA has become one of the most common mechanisms that B. pseudomallei uses to survive from the antibiotic treatment. We are currently developing real-time PCR assays to detect these mutations in B. pseudomallei in clinical settings. This project will not only empower clinicians with innovative tools to monitor drug susceptibility for B. pseudomallei in real-time, but also has a significant impact on alteration of treatment(s) for melioidosis.

97. MULTISYSTEMIC ENTEROCOCCUS INFECTION IN BROWN ANOLES FROM FLORIDA

Megan Caudill - College of Veterinary Medicine, University of Florida; **Tracey Logan** - College of Veterinary Medicine, University of Florida; **April Childress** - College of Veterinary Medicine, University of Florida; **Veronica Guzman-Vargas** - Florida Fish and Wildlife Conservation Commission; **Kevin M. Enge** - Florida Fish and Wildlife Conservation Commission; **Lisa Shender -** Florida Fish and Wildlife Conservation Commission; **Robert J. Ossiboff** - College of Veterinary Medicine, University of Florida

Beginning in June of 2019, numerous brown anoles (Anolis sagrei), an invasive lizard species in Florida, were observed with large, soft, subcutaneous masses of the head and body in the St. Petersburg area. Post-mortem examination of three animals, including cytologic, histologic, and electron microscopy evaluation, identified the presence of myriad coccoid bacteria surrounded by a prominent clear capsule with minimal associated granulomatous inflammation, which effaced normal tissues. PCR and sequencing of the 16S SSU gene and an internal transcribed spacer (ITS) region, revealed 100% nucleotide identity to a novel Enterococcus spp. first reported in 2014 as the cause of a severe, multisystemic, invasive infection in several species of endangered lizards

(geckos and skinks) on Christmas Island, an Australian external territory in the Indian Ocean. In previous investigations, further analysis of this organism has been hindered by an inability to grow the organism in standard culture media. In this study, the organism was successfully temporarily cultured on primary anole kidney cells. Interestingly, the bacteria were nearly always clustered tightly around host cells in culture, possibly suggesting a required symbiotic factor for their growth. Given the growing recognition of host species diversity and geographic distribution noted for this organism, there is potential concern for spread to native North American lizards, especially the green anole (Anolis carolinensis), whose population numbers have decreased due to introduced brown anoles.

98. OUTER MEMBRANE HOPANOIDS PLAY A ROLE IN BURKHOLDERIA MULTIVORANS MULTIDRUG RESISTANCE

Eric D. LoVullo - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Edward W. Yu** - Department of Pharmacy, College of Medicine, Case Western Reserve University, Cleveland, OH; **Herbert P. Schweizer** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Burkholderia multivorans is a Gram-negative opportunistic pathogen that is inherently resistant to many different classes of antibiotics. These bacteria belong to the B. cepacia complex (Bcc) whose members are responsible for causing severe respiratory infection in cystic fibrosis patients. Hopanoids are a class of membrane lipids that exist in certain bacterial genera whose physiological roles have not yet been fully elucidated but are thought to act like the eukaryotic sterols, e.g. cholesterol, to stabilize membranes and regulate their fluidity and permeability. It has already been established that hopanoid production is required for antimicrobial resistance in the Bcc bacterium B. cenocepacia. In Gram-negative bacteria hopanoids are synthesized into the inner membrane and must be transported to the outer membrane by the hopanoid biosynthesis-associated resistance nodulation cell division (RND) transporter, HpnN. Utilizing molecular genetic tools, we deleted the HpnN encoding gene, hpnN, from B. multivorans to investigate the role outer membrane hopanoids play in antimicrobial resistance. We

performed Biolog phenotypic microarrays on wild-type B. multivorans and ΔhpnN to assess the differences in antimicrobial sensitivities. We further defined some of these differences utilizing minimal inhibitory concentration assays. Our analyses confirmed that outer membrane hopanoids play a role in resistance to certain classes of antibiotics, e.g. tetracyclines. We are in the process of investigating the role of the second gene in the hpnNM operon, hpnM, which encodes a lipoprotein that is likely required for proper hopanoid insertion into the outer membrane.

99. RELATIONSHIP OF A MULTIPLEX MOLECULAR PNEUMONIA PANEL (PP) RESULTS WITH STANDARD MICROBIOLOGY LABORATORY RESULTS, HOSPITAL OUTCOMES AND CLINICAL VARIABLES

Kenneth Rand - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Stacy G. Beal** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Elizabeth Tremblay** - University of Florida; **Herb Houck** - Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida; **Kylie Lessard** - Department of Pathology, Immunology, and Laboratory Medicine, University of Florida; **Kartikeya Cherabuddi** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

The BioFire FilmArray Pneumonia Panel detects 15 common bacterial pathogens semi-quantitatively (copy # from 10^4 to 10^7), 3 atypical pneumonia bacteria (C. pneumoniae, Legionella, Mycoplasma), 8 viruses, and 7 antimicrobial resistance markers by multiplex PCR in about 1 hour in the laboratory. Consecutive bronchoalveolar lavage (BAL) specimens sent to the UF Health Shands clinical microbiology laboratory from June to September, 2018 were studied. Samples were frozen at -70^{o}C until testing could be carried out, hence results were not released to the treating providers. The multiplex PCR assay was compared with routine microbiology results (N=396) and corresponding clinical data available for 270 unique hospitalized patients. The PP was positive in 217/396 (55%) of patient samples, but culture was positive in only 137/396 (35%; p< 0.00001). The PP also detected more potentially pathogenic bacteria per BAL sample, i.e. 18 targets in 64 specimens with no growth in culture, 92 targets among 160 patients with normal flora only, and 121 additional

targets among 77 patients who were culture positive. Limiting the bacterial species to only those present on the panel, a total of 176 bacterial isolates were recovered in the 137 positive cultures; the PP detected 173 (98.3%) of these. By multiple independent one-way analysis of variance In the clinical population , the PP copy number was significantly related to temperature ($p < 0.001$), % PMNs in the BAL ($p < 0.001$), ICU days ($p < 0.006$), WBC reported on gram stain ($p < 0.00001$) and % with discharge coding of “Pneumonia” ($p < 0.001$). Conventional semi-quantitative culture results i.e. 1+, 2+, etc or >100,000 cfu/ml etc. was also statistically significantly related to outcome variables: ICU LOS ($p=0.016$), % coded as pneumonia ($p=0.013$), procalcitonin ($p=0.008$) and patients receiving antibiotics ($p=0.011$), although they did not entirely match the same variables as the PP copy number. Discussion and Conclusions 1.With the rapid in-laboratory turnaround time of ≈1 hour, the PP could have a major impact on appropriate antibiotic treatment decisions for potential pathogens that may cause pneumonia in intubated/ICU patients. 2.Because the test detects many more potential pathogens than culture alone, there might be concern for increasing antibiotic usage. However, in this high acuity patient group, >80% were already receiving antibiotics. 3.The PP semi-quantitative copy # shows a statistically significant association with clinical/outcome variables, at least as strong as that of conventional quantitation in the microbiology lab. 4.A negative result in conjunction with patient status could potentially help with antibiotic de-escalation.

100. SEROPREVALENCE OF MELIOIDOSIS AMONG SWINE IN TWO VIETNAMESE PROVINCES

Michael Norris - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Morgan Walker** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Andrew Bluhm** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Herbert P. Schweizer** - Department of Molecular Genetics and Microbiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Trinh Thanh Trung** - Vietnam National University, Hanoi; **Nga Vu Thi -** National Institute for Veterinary Research, Hanoi, Vietnam; **Ngoc Pham Thi** - National Institute of Veterinary Research, Hanoi, Vietnam; **Fred Unger** - International Livestock Research Institute, Vietnam; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Nguyen Thi Thu Hang** - National Institute of Veterinary Research, Hanoi, Vietnam

Background: Burkholderia pseudomallei is an environmentally mediated saprophytic pathogen that can cause severe disease in exposed individuals. It is well known that this organism survives in the moist soil environment in tropical regions worldwide and has been extensively studied in Thailand and Australia but melioidosis is now gaining recognition as a public and veterinary health issue in Vietnam. The contribution of animals to human disease is unknown, necessitating further investigation.

Methods: Swine sera were collected from two populations of swine, one grazing and one farmed, from two provinces in Vietnam. ELISAs utilizing B. pseudomallei capsular polysaccharide (CPS), outer polysaccharide (OPS), and HcpI protein were used to screen the serum samples. Positive samples were mapped to district or commune level. Seroprevalence calculations and global pig statistics were used to approximate number of swine exposures.

Results: Grazing pigs had higher levels of seropositivity compared to farmed pigs. Average swine seropositivity rates were 6.3%, higher than previously identified (~0.88%).

Conclusions: This initial swine sampling and serology work has identified a significant number of exposures and potential melioidosis infections occurring in swine in Vietnam. The contribution of swine to epidemiology and environmental persistence of melioidosis remains to be elucidated.

101. SWINE MELIOIDOSIS IN THAILAND: CASE REPORT

Treenate Jiranantasak - Department of Infectious Diseases and Immunology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Sawang Kesdangsakonwut** - Department of Pathobiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; **Apichai Tuanyok** - Department of Infectious Diseases and Immunology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Melioidosis, a neglected tropical disease, is endemic in Southeast Asia and Northern Australia. It is caused by Burkholderia pseudomallei, a Gram-negative bacillus. In endemic areas, the organism is common in the environment and most human and animal cases arise from exposure to contaminated soil and water. Melioidosis has been reported in a wide range of animal species and the most commonly affected livestock are goats, sheep, cattle and pigs. The retrospective study of melioidosis in animals in Thailand during 2006 – 2010 showed that the highest incidence was in goats, followed by incidence in pigs and cattle. Pigs have been reported as less susceptible to disease than sheep or goats. In pigs, melioidosis is frequently asymptomatic, often with lesions detected during routine abattoir inspection. In this study, melioidosis was detected in two pigs during routine farm inspection in the northern part of Thailand in 2016. Gross pathology showed diffuse white-yellowish abscesses in the lungs, liver, and lymph nodes. Histopathology revealed diffuse white blood cells infiltration, hemorrhage, and multiple granulomatous formations in tissues. The abscesses and lesions from all organs were negative on acid-fast stain, but cultured positive for B. pseudomallei. Molecular identification was performed by multiplex SYBR green real-time PCR detecting B. pseudomallei-specific sequences of the TTSS1-orf2 (type III secretion system), btfc-orf18 (the BTFC gene cluster target) and BPSS0120 (the YLF gene cluster target). The investigation has confirmed that the cause of the infection was B. pseudomallei by showing positive to TTSS1-orf2 and YLF genomic group, LPS type A. Multi-locus

sequence typing (MLST), a gold standard molecular subtyping technique of B. pseudomallei was performed by comparison of 7 housekeeping genes. MLST has further revealed that one strain had sequence type 164 (ST164) which had the same ST with the recent human case and soil samples in southern Thailand. This sequence type was also found in human cases in Malaysia. Another strain had sequence type 491 (ST491) which had the same ST with an isolate from a patient in Laos based on MLST database. In summary, the disease manifestations in pigs can range from acute to chronic with subclinical infection being common. Infection can be associated with single or multiple abscesses in lung, liver and associated lymph nodes. Real-time PCR potentially offers a faster and more reliable diagnosis of disease in endemic regions for melioidosis. The present cases showed the risk of zoonotic potential of B. pseudomallei in endemic area. Therefore, veterinarians treating animals from these areas should include melioidosis in differential diagnosis when performing necropsy. Furthermore, herdsmen, veterinarians, or abattoir workers should be aware of this risk of zoonotic transmission. Melioidosis is not currently part of the animal disease control program in Thailand, but its inclusion may now warrant review.

102. TAQMAN ASSAYS FOR SIMULTANEOUS DETECTION OF BACILLUS ANTHRACIS AND BACILLUS CEREUS BIOVAR ANTHRACIS

Diansy Zincke - Department of Geography, Emerging Pathogens Institute, University of Florida; **Michael Norris** - Department of Geography, Emerging Pathogens Institute, University of Florida; **Berzhan Kurmanov** - Department of Geography, Emerging Pathogens Institute, University of Florida; **W. Scott McGraw** - Department of Anthropology, The Ohio State University; **David J. Daegling** - Department of Anthropology, University of Florida; **John Krigbaum** - Department of Anthropology, University of Florida; **Hoang Thi Thu Ha** - Bacteriology Department, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, University of Florida; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, University of Florida

Anthrax is a worldwide zoonosis caused by the spore-forming bacteria Bacillus anthracis. Primarily affecting herbivores, human infections result from direct contact with contaminated animal products or through consumption of infected meat; the majority of human cases are cutaneous. The genetic near neighbor, Bacillus cereus biovar anthracis (Bcbva), causes anthrax-like disease in primates of west and central Africa due to the presence and expression of B. anthracis¬¬-specific virulence factors. While Bcbva anthrax-like infections have not been reported in humans, it is hypothesized that improved diagnostics will uncover cases in areas reporting Bcvba in bush meat or livestock. No single assay thus far exists for the prompt and concurrent detection of these two pathogens producing classical anthrax and anthrax-like illness. Here we describe new TaqMan multiplex PCR assays for the simultaneous identification of B. anthracis and Bcbva. The assays are designed to amplify Ba-1, capB, and lef markers in B. anthracis and genomic island IV (GI4), capB, and lef in Bcbva. A four-dye format was developed for the QuantStudio 7 platform that specifically detected unique pathogen markers. The assay was also standardized for the LightCycler 2.0 platform, which remains common across diagnostic laboratories worldwide. The assays were validated using a panel of 26 diverse B. cereus sensu lato strains including B. cereus, B. thuringiensis, B. anthracis, and Bcbva. All B. anthracis isolates known to be positive for capB, lef and Ba-1 markers produced clear and reproducible amplification curves of all three

markers. The GI4 marker was specific to Bcbva and was not amplified in any other strains tested. The five different Bcbva isolates tested showed strong amplification of capB, lef and GI4 while failing to amplify the B. anthracis-specific Ba-1 marker. The specificity of capB and lef was illustrated with capB- and lef- negative strains. Specifically, the various B. anthracis capB negative strains tested produced strong lef and Ba-1 signals whereas only Ba-1 and capB were amplified in lef-negative strains. Our work here illustrates the development of TaqMan assays for detection of both B. anthracis and Bcbva. The ability to concurrently determine Ba-1, GI4, lef, and capB status will be useful in areas where these pathogens overlap geographically, currently central and western Africa. These assays are now also integrated into national diagnostics in Vietnam. In addition, the assays can be readily adapted for the sole detection of B. anthracis, pXO1 and pXO2, in areas where Bcbva is improbable.

103. THERAPEUTIC MONITORING OF BETA-LACTAMS AND ASSOCIATED THERAPY OUTCOMES IN CRITICALLY ILL PATIENTS

Mohammad Al-Shaer - Department of Pharmacotherapy and Translational Research, Emerging Pathogens Institute, College of Pharmacy, University of Florida; **Eric Rubido** - Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida; **Kartikeya Cherabuddi** - Department of Infectious Disease, College of Medicine, University of Florida; **Kenneth Klinker** - Merck; **Charles Peloquin** - Department of Pharmacotherapy and Translational Research, Emerging Pathogens Institute, College of Pharmacy, University of Florida

Background: In the intensive care unit (ICU), early and appropriate antimicrobial therapy is important to lower infection-related mortality. Our aim was to assess whether achieving beta-lactam (BL) free concentration above the minimum inhibitory concentration 100% of the time (fT>MIC) is associated with positive outcomes in critically ill.

Methods: This retrospective study was conducted in critically ill patients at UFHealth Shands Hospital from 2016 to 2018. Adult patients who received BL therapy and had drug concentration measured were included. Data collected included demographics, lab results, BL regimens and concentrations, source of infection, cultures and susceptibilities,

mortality, length of stay, resistance acquisition for 30 days, and clinical outcome at end of therapy. Multivariate regression and time-to-event (TTE) analyses were performed.

Results: 471 patients were included. Clinical cure in 71%, microbiologic eradication in 52%, and new resistance to BL received developed in 6% of patients. Hospital and 30-day mortality were 17% and 15%, respectively. fT>MIC and fT>4xMIC were associated with clinical cure (p=0.0303), microbiologic eradication (p=0.0476), and suppression of resistance (p=0.0043). Delay in measuring BL concentration was associated with less clinical cure (p=0.0072), longer ICU stay (p<0.0001), and higher mortality (p=0.0387). In the TTE analysis, patients with 100% fT>MIC had significantly shorter ICU stay (p=0.0297). Patients who had clinical cure and microbiologic eradication had plasma drug concentrations measured earlier (p=0.0025 and 0.0254, respectively).

Conclusions: This study highlights the importance of early measurement of BL plasma concentration in ICU as both fT>MIC and days until first concentration measurement were associated with improved clinical outcomes.

104. APPLICATION OF VIRUS-BLOCKING PEPTIDES TO MITIGATE VIRUS BURDEN IN THE HONEY BEE

Ya Guo - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Bryony C. Bonning** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Colonies of the western honey bee, Apis mellifera, have been severely impacted in recent years by a wide range of stressors. The causes of bee declines are multifactorial, but Varroa mites and associated viruses are among the most serious threats to honey bee health. The picture that has emerged over the course of a decade of research is that virus load plays an important part in weakening of colonies prior to their demise. The iflavirus, Deformed wing virus (DWV) and the dicistrovirus, Israeli acute paralysis virus (IAPV) are of particular concern in relation to colony losses. DWV and IAPV are hypothesized to invade midgut epithelial cells of honey bees by receptor-mediated endocytosis, but the specific molecular mechanisms of this process have not been revealed. By feeding adult honey bees on a phage display library, peptides that bind the honey bee midgut were identified. The most enriched peptide, Bee midgut Binding Peptide (BBP2.1), shared 75% and 85% identity with a region of the DWV and IAPV capsid proteins, respectively. This region of the capsid protein is likely to be instrumental in virus interaction with the honey bee gut receptor. BBP2.1 and the two similar virus-derived sequences, peptides BBP2.1DWV and BBP2.1IAPV were cloned and fused with mCherry for further analysis. Peptide binding to honey bee gut brush border membrane vesicles (BBMV; enriched in membrane proteins) was confirmed in vitro by pull-down assay with all three peptide-mCherry fusion proteins binding to BBMV. Competition assays showed that all three peptides compete with both IAPV and DWV virions for binding to honey bee gut-derived BBMV suggesting that the three peptides and the two viruses bind to the same protein or proteins. Preliminary data showed that ingestion of BBP2.1 reduced the movement of both IAPV and

DWV from the honey bee gut into the body, supporting the utility of these peptides for peptide-mediated interference with infection. The practical application of such virus-blocking peptides toward suppression of virus infection, and reduction in both virus load and virus-associated mortality will be explored.

105. CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 - 2019

Justine L. Smith - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Ashley G. Hodge** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Hannah M. Barber** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Sydney L. Cottingham** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Olivia Goodfriend** - Department of Wildlife Ecology and Conservation, University of Florida; **Katherine A. Sayler** - Department of Wildlife Ecology and Conservation, University of Florida; **Julia Loeb** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Jeremy P. Orange** - Department of Wildlife Ecology and Conservation, University of Florida; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

White-tailed deer (Odocoileus virginianus) farming is an emerging agricultural industry in Florida. Bacterial infections and viral hemorrhagic diseases cause high mortality in fawn and yearling deer, and are a source of significant production loss among Florida deer farmers. Before management can be improved and properly implemented, the causality of death in the farmed herds must be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of farmed white-tailed deer that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes of death. From 2017 to 2019, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Both necropsy and owner-sampled tissues

were tested for hemorrhagic disease using qPCR, and were subjected to additional microbial culture, histopathology analysis, and parasite identification as necessary to determine probable cause of death. Of the 128 deceased farmed white-tailed deer sampled in 2017, 39% of deaths were associated with bacterial infection, 44% were attributed to viral hemorrhagic disease, and 17% of deaths were due to other or undetermined causes. Of the 152 animals sampled in 2018, 40% of deaths were attributed bacterial infection, 49% were associated with hemorrhagic disease virus, and the remaining 11% of animals sampled died of other or unknown causes. Of the 126 animals sampled throughout 2019, 30% of deaths were attributed to bacterial infection, 42% were attributed to hemorrhagic disease virus, and the final 28% of animals sampled died from unknown or other causes. Viral hemorrhagic diseases and bacterial infections are major sources of mortality in farmed white-tailed deer. These data provide white-tailed deer farmers with insight on how to improve management practices, thereby improving herd health and reducing mortalities.

106. CHARACTERIZATION OF A NOVEL BUNYAVIRUS ISOLATED FROM MORIBUND FARMED AND FREE-RANGING FRESHWATER TURTLES IN FLORIDA

Thomas Waltzek - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Brian A. Stacy** - University of Florida; **Robert J. Ossiboff** - College of Veterinary Medicine, University of Florida; **Nicole Stacy** - College of Veterinary Medicine, University of Florida; **William A. Fraser** - Florida Department of Agriculture and Consumer Services; **Shipra Mohan -** Florida Department of Agriculture and Consumer Services; **Thais C. S. Rodrigues** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Veronica Guzman-Vargas** - Florida Fish and Wildlife Conservation Commission; **Lisa Shender** - Florida Fish and Wildlife Conservation Commission

In 2006, increased mortality was observed on a Florida farm rearing softshell turtles (Apalone ferox; ST). A moribund ST was submitted to the Bronson Animal Disease Diagnostic Laboratory in Kissimmee, FL for

diagnostic evaluation. Tissue homogenates inoculated onto confluent monolayers of Terrapene Heart (TH-1) cells resulted in extensive cytopathic effects (CPE). The supernatant was diluted and passaged onto fresh TH-1 cells with the same CPE observed. The supernatant was processed for negative stain electron microscopy and abundant spherical virus particles displaying prominent surface projections were observed. An infected TH-1 culture was shipped to the University of Florida's Wildlife and Aquatic Veterinary Disease Laboratory in Gainesville, FL for genetic characterization. An RNA extract from the infected culture was used to generate a cDNA library for sequencing on a MiSeq sequencer. BLASTX searches of assembled contigs identified the tripartite genome of a novel bunyavirus including the viral genes encoding the RNA-dependent RNA polymerase (RdRp), glycoproteins, non-structural protein, and nucleoprotein. PCR confirmed the three turtle bunyavirus (TBV) segments were amplifiable in infected TH-1 cultures, but not in the TH-1 cell line. A PCR targeting the TBV RdRp was developed to screen freshwater turtle samples collected by staff of the Florida Fish and Wildlife Conservation Commission as part of an ongoing epizootic (2018-19). 7/7 STs and 2/3 peninsular cooters (Pseudemys peninsularis; PC) tested positive (35/36 and 4/8 tissue samples, respectively). Three additional STs sampled from unrelated cases tested negative by PCR (0/7) and the aforementioned PCR-negative (0/4) PC was determined to have died from trauma. We plan to develop a RNAscope in situ hybridization assay and a TaqMan quantitative PCR assay to assist in elucidating the tissue distribution, viral load, and pathogenesis of the TBV. An experimental challenge study will be conducted to determine the role of the TBV in disease of freshwater turtles.

107. CHARACTERIZATION OF A NOVEL MARINE ALPHAVIRUS ISOLATED FROM A STRANDED HARBOUR PORPOISE (PHOCOENA PHOCOENA) FROM ALASKA

Thais C. S. Rodrigues - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Ole Nielsen** - Department of Fisheries and Oceans Canada; **Vsevolod L. Popov** - University of Texas Medical Branch; **Kathleen A. Burek-Huntington** - Alaska Veterinary Pathology; **David Rotstein** - Marine Mammal Pathology Services; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

The family Togaviridae comprises several significant human and veterinary mosquito-borne pathogens, including Chikungunya virus and Western equine encephalitis virus. Only one togavirus (genus Alphavirus) has been previously identified from a marine mammal, the Southern elephant seal virus (SESV), recovered from the louse (Lepidophthirus macrorhini) parasitizing apparently healthy Southern elephant seals (Mirounga leonina). Herein we report the ultrastructural and phylogenomic characterization of a novel marine togavirus, the first isolated from a cetacean, an Alaskan harbour porpoise (Phocoena phocoena). The animal displayed ulcerative dermatitis and a skin sample was processed for virus isolation on Vero.DogSLAMtag cells. Cytopathic effects (CPE) were observed approximately 20 days post-infection. An infected flask of Vero.DogSLAMtag cells displaying extensive CPE was prepared for transmission electron microscopy and revealed typical alphavirus particles ~60 nm in diameter budding from both plasma and vacuolar membranes of infected cells. Virus nucleocapsids ~25 nm in diameter were seen lining up along the cytoplasmic side of these membranes before budding. A next-generation sequencing approach was used to determine the complete genome (11,425 bp) of the Alaska harbor porpoise alphavirus (AHPV). Phylogenetic analysis based on the complete protein coding sequence supported the AHPV as the sister species to the SESV, forming a marine mammal alphavirus clade separate from the recognized alphavirus complexes. Genetic comparison of the protein coding sequence of the AHPV to other alphaviruses demonstrated amino acid (aa) identities ranging from 42.1-67.1%, with the highest identity to

the SESV. Based on its genetic divergence, we propose the AHPV represents a novel alphavirus species, pending formal proposal to and ratification by the International Committee on Taxonomy of Viruses. The ecological and genetic characteristics of the AHPV and the SESV also suggest they represent a novel complex within the genus Alphavirus, which we propose to be named the Marine Mammal Virus Complex. The role of the AHPV in the associated harbor porpoise cutaneous pathology, if any, remains unclear. Further research is needed to determine AHPV's route(s) of transmission and potential vectors, host range, prevalence, and pathogenicity in cetaceans including harbour porpoises.

108. CHARACTERIZATION OF A PERIBUNYAVIRUS ISOLATED FROM LARGEMOUTH BASS (MICROPTERUS SALMOIDES)

Thomas Waltzek - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Eric Leis** - U.S. Fish and Wildlife Service; **Ryan Katona** - U.S. Fish and Wildlife Service; **Terry Fei Fan Ng** - University of Georgia; **Eric Delwart -** University of California; **Marisa Barbknecht** - University of Wisconsin–La Crosse; **Kelly Rock** - University of Wisconsin–La Crosse; **Michael A. Hoffman** - University of Wisconsin–La Crosse

The order Bunyavirales includes 12 viral families that possess enveloped nucleocapsids with multipartite (2–8 segments), single-stranded, negative-sense or ambisense RNA genomes. Bunyaviruses include notable human (e.g., hantaviruses and Crimean-Congo hemorrhagic fever virus), veterinary (e.g., Rift Valley fever virus), and plant (e.g., tomato spotted wilt virus) pathogens. They may be vectored through blood-sucking (e.g., mosquitoes or ticks) or sap-sucking (e.g., thrips) arthropods (arboviruses) and rodents (rodoviruses). Herein, we report the complete genome sequencing of the first fish peribunyavirus determined using a next-generation sequencing approach. In April of 2009, as part of the National Wild Fish Health Survey, 30 largemouth bass were netted from Pool 10 of the upper Mississippi River near Prairie du Chien, Wisconsin. Kidney, spleen, and swim bladder tissues were pooled and processed for virus isolation. Upon infection, the BF-2 cells lost their differentiated shape as they condensed and detached from the plate, occasionally appearing

refractile. Transmission electron microscopy revealed abundant spherical virus particles (≈85 nm in diameter), each with a prominent globular fringe indicative of an enveloped virus. Next-generation sequencing recovered three contigs with greatest BLAST scores to the large (L), medium (M), and small (S) segments of members of the genus Orthobunyavirus within the family Peribunyaviridae. Maximum Likelihood phylogenetic analyses based on the concatenated amino acid (aa) sequence alignments of RNA-dependent RNA polymerase (RdRp), glycoprotein precursor polyprotein, and nucleoprotein supported the largemouth bass bunyavirus (LBBV) as the sister group to members of the family Peribunyaviridae. Genetic analysis based on the aa sequence alignment of the RdRp showed sequence identity of the LBBV to other bunyaviruses ranged from 23.4%–30.4% (highest identity to Herbert virus). The LBBV represents the first peribunyavirus characterized from an ectothermic vertebrate host. The isolation of the LBBV offers a unique opportunity to determine its role in disease and future challenge studies are planned. The host range of the LBBV, route(s) of transmission including potential aquatic vectors (e.g., blood-sucking crustacean parasites or leeches), and the prevalence within wild and cultured stocks of largemouth bass requires further investigation.

109. CONSECUTIVE REPORT OF A MEGALOCYTIVIRUS INFECTION IN AQUACULTURED ALBINO RAINBOW SHARKS (EPALZEORHYNCHOS FRENATUS)

Samantha Ayumi Koda - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Pedro H. de Oliveira Viadanna** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Deborah Pouder** - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Roy Yanong** - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

The family Iridoviridae consists of double-stranded DNA viruses that infect invertebrates, fish, amphibians, and reptiles. Members of the genus Megalocytivirus (subfamily Alphairidovirinae) are known to infect >150 species of marine and freshwater ornamental and food fish species around the world. Megalocytiviruses (MCVs) are subdivided into three genotypes: infectious spleen and kidney necrosis virus (ISKNV), red seabream iridovirus, and turbot reddish body iridovirus. MCVs of the genotype ISKNV are notable pathogens negatively impacting the global production of ornamental fishes. In September 2018, a chronic low-level mortality event in farm-raised juvenile and broodstock albino rainbow sharks Epalzeorhynchos frenatus was observed. The non-specific clinical signs included lethargy, increased gilling, and abnormal position in the water column. In October 2019, on a second fish farm, a similar low-level mortality event was observed in juvenile albino rainbow sharks exhibiting similar non-specific clinical signs. Bacteriological and parasitological investigations in the 2019 case revealed a moderate and consistent load of cutaneous ectoparasites (Trichodina sp.) and digenean metacercariae in the gills of both moribund and normal appearing fish. Histopathological examination revealed microscopic lesions consistent with MCV infection including the presence of cytomegalic cells (i.e., basophilic intracytoplasmic inclusions) in internal tissues of the 2018 case. In both years, internal tissue homogenates from clinical fish were inoculated onto the grunt fin cell line and cytopathic effects (e.g. refractility, cytomegaly)

were observed three to six days post-infection. Extracted DNA from matching tissue homogenates were positive using a MCV-specific quantitative PCR. DNA samples from the 2018 and 2019 cases were used to generate DNA libraries for sequencing on an Illumina MiSeq sequencer. The resulting sequence data recovered the complete genomes of nearly identical ISKNV strains in the 2018 and 2019 cases. Similarly, a Maximum Likelihood phylogenetic analysis based on the nucleotide alignment of 18 MCV full genomes supported the rainbow shark MCVs as each other's closest relatives within the ISKNV genotype. In both cases, only the albino rainbow sharks displayed disease despite sharing the same re-circulated water source with several other ornamental fish species. Discussions with the farm manager in the 2019 case revealed they had received rainbow shark stocks from the farm that had experienced the MCV disease episode in 2018. The detection of ISKNV in this highly valued ornamental fish species underscores the need for improved biosecurity measures and mitigation strategies to protect the ornamental fish industry from MCVs.

110. DOES ALCOHOL CONSUMPTION PREDICT FUTURE HIV VIRAL SUPPRESSION? A PROSPECTIVE COHORT STUDY AMONG PERSONS LIVING WITH HIV IN FLORIDA

Veronica Richards - Department of Epidemiology, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida; **Rebecca Fisk** - Department of Epidemiology, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida; **Emma C. Spencer** - Florida Department of Health; **Robert Cook** - Department of Epidemiology, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida

Background: Among persons living with HIV (PLWH), heavy alcohol consumption is associated with a lower likelihood of achieving undetectable viral loads (VL, <200 copies/mL blood). However, much of the evidence investigating this has been cross-sectional. To meet the U.S. goal of ending the HIV epidemic by 2030, it is critical to understand how alcohol consumption affects viral suppression over longer time-periods. This study aims to describe VL distribution 2 years post-baseline across drinking categories to better understand how alcohol consumption predicts future VL.

Methods: Between October 2014-December 2018, 932 PWLH were recruited into the Florida Cohort, which uses self-administered questionnaires to collect data on demographics and alcohol consumption. Drinking categories were heavy drinking (>7 drinks/week for women, >14 drinks/week for men), binge drinking (>3 drinks/occasion for women, >4 drinks/occasion for men), low-level drinking (not heavy or binge) and no drinking. Questionnaire data were linked to VL from the Enhanced HIV/AIDS Reporting System, managed by the Florida Department of Health. Analysis was limited to participants who had a VL available at baseline (up to 90 days before) and 2 years after (up to 90 days after).

Results: The sample consisted of 806 PLWH (63% 45 years or older, 65% male, 56% Black). Most participants had consistently undetectable VL (65%), 10% had consistently detectable V L, 17% transitioned from detectable to undetectable VL, and 8% transitioned from undetectable to detectable VL . Furthermore, 676 PLWH provided data on alcohol consumption (10% heavy drinking, 28% binge drinking, 39% low-level drinking, and 23% no drinking in the past year). Persons with heavy drinking (48%) at baseline had lower proportions of consistent undetectable VL compared to persons with binge drinking (64%), low-level drinking (65%), and no drinking (71%). Fifteen percent of persons with heavy drinking had consistently detectable VL, compared to 9% with binge drinking, 11% with low-level drinking, and 8% with no drinking. Persons with heavy drinking experienced more overall VL transitions, with 27% moving from detectable to undetectable and 9% moving from undetectable to detectable, compared to binge drinking (16% and 11%, respectively), low-level drinking (18% and 6%, respectively), and no past year drinking (16% and 6%, respectively).

Conclusions: Baseline drinking status may predict the likelihood of achieving undetectable VL 2 years into the future. Persons with heavy drinking may be less likely to achieve consistently undetectable VL. Integration of alcohol-reduction interventions with HIV care may improve HIV health outcomes among PLWH who drink heavily.

111. ENRICHMENT OF TRANS-CLEAVAGE ACTIVITY OF CAS12A WITH MODIFIED CRRNA ALLOWS FOR AMPLIFIED NUCLEIC ACID DETECTION

Long Nguyen - Department of Chemical Engineering, College of Engineering, University of Florida; **Brianna Smith** - Department of Chemical Engineering, College of Engineering, University of Florida

The CRISPR/Cas12a RNA-guided complex has the potential for nucleic acid detection due to its ability to indiscriminately cleave ssDNA once bound to a target double-stranded DNA (dsDNA). However, current CRISPR/Cas12a systems are limited to a nanomolar detection limit without an amplification step. In this study, we extended the 3'- or 5'-ends of the crRNA with different lengths of DNA, RNA, and phosphorothioate DNA, and we observed amplified Cas12a trans-cleavage activity as high as 3.5-fold compared to the wild type crRNA on the target dsDNA eGFP fragment. Employing this phenomenon, we developed a detection assay with optimized conditions that enables us to detect nucleic acids with high sensitivity on a femtomolar scale without an amplification step based on limit of detection analysis. We then applied this system to detect low copies of HIV RNA without any target amplification. The findings hold promise for highly sensitive nucleic acid detection that could be used for the early diagnosis of various infectious diseases.

112. ESTABLISHING A HIGH SCHOOL VACCINATION PROGRAM TO IMPROVE VACCINE UPTAKE FOR RECOMMENDED ADOLESCENT VACCINES INCLUDING HUMAN PAPILLOMA VIRUS (HPV), MENINGOCOCCAL ACWY (MCV), HEPATITIS A (HEP A) AND MENINGITIS B (MEN B)

Kathleen Ryan - Department of Pediatrics, Eric Friedheim Tourism Institute, College of Medicine, University of Florida; **Robert Lawrence** - Department of Pediatrics, College of Medicine, University of Florida; **Paul Myers** - Florida Department of Health; **Brad Caron** - Florida Department of Health; **Amit Rawal** - Avnee Foundation; **Kayla Talebi** - Department of Public Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Eric Richardson** - Department of Health Outcomes and Policy, Institute for Child Health Policy, University of Florida; **Stephanie Staras** - Department of Health Outcomes and Policy, Institute for Child Health Policy, College of Medicine, University of Florida

Background: Adolescent vaccination rates for HPV and MCV remain below Healthy People 2020 goals. In addition, vaccination rates for Men B remain low due in part to lack of unawareness of Men B vaccine and to the category B recommendation. Despite recommendation for Hep A vaccination beginning at one year of age, many adolescents remain unvaccinated since Hep A is not required for school entry in many states. Increasing adolescent vaccination rates using traditional physician office-based methods has proven to be challenging. Therefore, we established a public high school vaccination program in Alachua County, Florida for 11th and 12th graders offering HPV, MCV, Men B and Hep A vaccines in 2018 and 2019. The program was modeled after the well-established and successful school-located influenza vaccination program (Control Flu) that has been in operation in Alachua County since 2009.

Methods: This is a collaboration between the Alachua County Schools, the Florida Department of Health in Alachua County, the Medical Reserve Corps and the University of Florida Department of Pediatrics and the Avnee Foundation. . Vaccines were offered at seven high schools (2018 – 3996, 2019 -3207 11th &12th graders). At each school, two clinics were held one month apart to allow for second dosing of HPV and Men B vaccine if needed. Schools distributed consent forms to parents one month prior to clinics. Vaccination records and doses needed were

verified for each student in the Florida State Vaccination registry (FLShots). Children who had parent consent were given vaccines during lunch hours or pulled from classes. Vaccine administration expenses were billed to the child's insurance.

Results: In 2018, 394 total doses of vaccine were given (147 Men B, 81 HPV, 101 MCV, 65 Hep A). In 2019, 598 total doses were given (298 Men B, 130 HPV, 98 MCV, 63 Hep A). During both years, Men B was most frequently given (37% and 51% of vaccines) followed by HPV (20.5% and 22%). Approximately 50% more doses were given to students covered by commercial insurance than students receiving Vaccine for Children (VFC) vaccine.

Conclusion: The vaccination program was well received and no significant adverse events occurred. Bundling . Establishing a school-located adolescent vaccination program can enhance uptake of recommended adolescent vaccines and should be considered as another avenue for increasing immunization rates in this age group.

113. GENOME CHARACTERIZATION OF CETACEANPOX VIRUS FROM A MANAGED INDO-PACIFIC BOTTLENOSE DOLPHIN (TURSIOPS ADUNCUS)

Thais C. S. Rodrigues - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Arvind Varsani** - Arizona State University; University of Cape Town; **Grant McFadden** - Arizona State University; **Adam M. Schaefer** - Florida Atlantic University; **Gregory D. Bossart -** University of Miami, Georgia Aquarium; **Carlos H. Romero** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Cetaceanpox viruses (CePVs) are associated with a cutaneous disease in cetaceans often referred to as "tattoo" lesions. To date, only partial genomic data are available for CePVs, and thus, they remain as unclassified members of the subfamily Chordopoxvirinae within the family Poxviridae. Analyses of the partial DNA polymerase and DNA topoisomerase I genes have suggested the presence of at least two

groups: CePV-1 infections in odontocetes and CePV-2 infections in mysticetes. Herein, we describe the first complete CePV genome sequenced from the tattoo lesion of a managed Indo-Pacific bottlenose dolphin (Tursiops aduncus), an odontocete, using next-generation sequencing. The complete genome of the cetaceanpox virus from T. aduncus (CePV-TA) was determined to be 121,769 bp with an A+T content of 71.5 %. The inverted terminal repeats (ITRs) at the 5' and 3' ends of the genome included 3666 bp and the terminal hairpin palindromic sequences were determined. The CePV-TA was determined to encode 120 proteins, including eight unique genes and five genes predicted to function as immune-evasion genes. CePV-TA-4 and CePV-TA-117, located in the ITRs, encode apoptosis regulator M11L like proteins; CePV-TA-20 and CePV-TA-21 encode double-stranded (ds) RNA binding IFN resistance/protein kinase (PKR) inhibitor proteins; and CePV-TA-113 encodes a chemokine binding protein. Comprehensive genetic analyses using a suite of core poxvirus-conserved genes supported CePV-TA as the sister group to a large clade that includes members of the genera Centapoxvirus, Orthopoxvirus, and clade II poxviruses (i.e., Cervidpoxvirus, Suipoxvirus, Capripoxvirus, Leporipoxvirus, and Yatapoxvirus). Our genetic and phylogenetic analyses reinforce previous proposals in support of the establishment of a new chordopoxvirus genus for poxviruses from cetaceans (i.e., proposed cetaceanpoxvirus). CePV-TA could serve as the type species (Cetaceanpox virus 1) pending formal proposal to and ratification by the International Committee on Taxonomy of Viruses. The determination of the CePV-TA genome facilitated the first analyses to unravel the evolutionary relationship and taxonomy of CePVs. The availability of the CePV-TA genome sequence will assist future efforts in determining variations and dynamics of CePVs in varied cetacean hosts including mysticetes. The complete CePV-TA genome will also enable the development of more specific molecular diagnostic assays (e.g., in situ hybridization) to assist in the surveillance of CePVs and better define their role in cetacean disease.

114. HIV+ ADULTS WITH CURRENT AUD, AND ELEVATED CEREBRAL MYO-INOSITOL CONCENTRATIONS PREDICT REDUCED COGNITIVE FUNCTION OVER 12-MONTHS

Vaughn Bryant - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Eric Porges** - Department of Clinical and Health Psychology, College of Public Health and Health Professions, University of Florida; **Robert Cook** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Ronald Cohen** - Department of Clinical and Health Psychology, College of Public Health and Health Professions, University of Florida

Purpose: To examine the relationship between Human Immunodeficiency Virus (HIV), alcohol use disorder (AUD), and magnetic resonance spectroscopy (MRS) markers of neural injury, on cognitive function.

Introduction: The prevalence of AUD among HIV+ adults is nearly double that of the general population. Both HIV and AUD are associated with cognitive deficits in the domains of executive function, processing speed, working memory, learning, memory, and fine motor dexterity, and also have detrimental effects on brain structure and function. Proton MRS provides a unique, sensitive and non-invasive measure of cerebral metabolic integrity. Common proton MRS markers include N-acetylaspartate (NAA), creatine (CR), choline (Cho), and myo-inositol (mI). Recent proton MRS methods also enable the measurement of GABA concentrations. Past studies indicate elevated levels of mI, Cho and Cr in frontal and basal ganglia regions, along with reduced levels of NAA, among HIV+ adults. Most of these studies have been cross-section, and none have examined whether baseline MRS abnormalities in the context of HIV and AUD predict reduced cognitive functioning over time, the goal of the current study.

Method: Sixty-four participants in a National Institute on Alcohol Abuse and Alcoholism (NIAAA) sponsored study of the effects of heavy alcohol use and aging on neurocognitive and brain functioning were assessed at baseline and 12-month follow-up. Alcohol use disorder diagnosis was assessed by the SCID-4. Repeated measures MANCOVA was conducted, controlling for age, total gray matter volume and subcortical white matter volume.

Results: There was a significant HIV x AUD x time interaction [F(12, 94) = 1.88, p = .045], with univariate analyses indicating that the effect was primarily within the domain of processing speed [F(2, 58) = 3.836, p = .028]. Estimated marginal means plot indicated that HIV + current AUD performed most poorly. There was a mI/CrPCr x time interaction [F(1, 46) = 2.3, p = .05], with univariate measures noting an effect specific to memory [F(1, 46) = 10.65, p = .002].

Conclusion: Pathological alcohol consumption exacerbates HIV related effects on processing speed. MI in the basal ganglia may be a sensitive predictor of memory change and may be a marker of neural injury.

115. IDENTIFICATION OF DIVERGENT SERPENTOVIRUSES IN FREE-RANGING INVASIVE PYTHONS IN SOUTHERN FLORIDA

Steven Tillis - Department of Veterinary Medical Sciences, College of Veterinary Medicine, University of Florida; **Melissa A. Miller** - Florida Fish and Wildlife Conservation Commission; **Jillian Josimovich** - U.S. Geological Survey, Fort Collins Science Center; **Natalie Claunch** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Ian Bartoszek** - Conservancy of Southwest Florida; **Arik Hartmann -** US National Park Service; **John Humphrey** - US Department of Agriculture, Animal and Plant Health Inspection Service; **Bryan Kluever** - US Department of Agriculture, Animal and Plant Health Inspection Service; **Robert N. Reed** - U.S. Geological Survey; **Christina M. Romagosa** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida,

Established invasive pythons in southern Florida, such as the Burmese python (Python bivittatus), have significantly impacted native ecosystems. Aside from direct predation and competition, invasive species can also introduce nonnative pathogens that can negatively affect native species. The genus Serpentovirus (order Nidovirales) is composed of positive-sense RNA viruses primarily found in reptiles. Some serpentoviruses, such as shingleback nidovirus, are associated with mortalities in wild populations, while others, including ball python nidovirus and green tree python nidovirus, can be a major cause of disease and mortality in captive animals. To determine if serpentoviruses were present in invasive pythons in south Florida, oral swabs were collected from both long-term captive and free-ranging in situ pythons.

Swabs were screened for the presence of serpentoviruses by reverse transcription PCR. Sanger sequencing of PCR amplified products identified 34% of pythons tested (n=152) were positive for multiple divergent sequences during initial testing, circulating in the invasive pythons across their range. Locale within their range was statically significant in both the sequences of viruses circulating (p=3.801e-12) and in prevalence of virus (p=0.17). Clinical signs and postmortem lesions consistent with Serpentovirus infection were observed in a subset of sampled pythons. Testing of native snakes (n=201, 18 species) in part of the python range found no evidence of spillover from python viruses. However this does not fully encompass the sequence diversity of viruses seen across the range of wild python populations, and the potential for spillover to indigenous herpetofauna is of concern and warrants further investigation.

116. NEGATIVE CONSEQUENCES OF ALCOHOL USE AMONG PEOPLE LIVING WITH HIV ENROLLED IN A COMMUNITY-BASED COHORT IN FLORIDA

Rebecca Fisk - Department of Epidemiology, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida; **Veronica Richards** - Department of Epidemiology, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida; **Robert Leeman** - Department of Health Education and Behavior, College of Health and Human Performance, SHARC Center for Translational HIV Research, University of Florida; **Christa Cook** - College of Nursing, University of Central Florida; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, SHARC Center for Translational HIV Research, University of Florida

Purpose: Among people living with HIV (PLWH), alcohol use has a deleterious effect on antiretroviral adherence and viral suppression. Alcohol use can also have negative impacts on finances and relationships, which may be as meaningful as HIV-related outcomes. It is important to understand the type and magnitude of consequences of drinking among PLWH. The objective of this study is to identify the most common consequences among PLWH in Florida and describe factors associated with experiencing more alcohol-related consequences. This information

will help identify groups who may benefit more from interventions to reduce alcohol use and improve HIV-related health outcomes.

Methods: Data were collected from PLWH in the Florida Cohort, a community-based cohort study (65% male, 55% African-American). Participants were recruited from seven sites across Florida between 2014 and 2016. Participants were included in this analysis if they drank at least monthly in the past year (n= 404). Self-reported consequences in the preceding 3 months were assessed at baseline using a dichotomized Short Inventory of Problems Revised (SIP-R) with possible scores ranging from 0 to 15. Consequences were ranked by reported frequency. Wilcoxon signed rank tests and Spearman correlations were used to evaluate associations of the total SIP-R score with demographics, mental health, socioeconomic status, alcohol, and drug use. A multivariable linear regression model was developed using significant variables from the bivariate analyses.

Results: Mean SIP-R score was 3.4 (range 0-15), and 43% reported zero consequences. The most common consequences were taking foolish risks (37.9%) and doing something impulsive that they regretted (36.8%). Although less common, 11.7% reported getting into an accident. In bivariate analyses, higher SIP-R scores were significantly ($p<0.05$) associated with lower education, depression, anxiety, drug use within the last year, homelessness, average drinks per week in the last year, and largest amount of alcohol consumed in the last month. In multivariable analysis, only average drinks per week, anxiety, and homelessness remained significantly associated with greater SIP-R scores.

Conclusions: In addition to those who drink heavily, persons who are homeless or who have anxiety should be given special attention when designing interventions to reduce alcohol use. Reducing alcohol use may help these individuals achieve optimal antiretroviral adherence and viral suppression, which will help improve individual health outcomes and reduce HIV transmission in the community.

117. REMEMBERING POLIO IN AMERICA

Nina Stoyan-Rosenzweig - University of Florida

Polio, now mostly eliminated from the American landscape, held a very different position in the early-mid 20th century. It became epidemic, striking children especially, and its appearance in American communities especially in the summer created a great deal of fear. Polio sufferers also, to some extent, faced fear and exclusion as people around them did not understand how infectious they might be. However, given the people who were infected- mostly children, and in the 1940s, the US president, the support for finding a cure or vaccine resulted in significant focus on raising funds for research. With the development of successful vaccines, the disease has mostly vanished and the last generation to have had polio are now elderly. In order to promote collective memory and understanding of the polio impact, the UF libraries, throughout the Library Press, are publishing a unique journal recounting Edna Hindson's experience with polio in Lake City in the 1940s. This poster will discuss the history and impact of polio, the upcoming book, and explore how fear of infection vanished with the disease.

118. ROLE OF NOROVIRUS AND MEMBRANE VESICLES FROM COMMENSAL BACTERIA INTERACTION IN HOST CELL INFECTION AND DISEASE

Sutonuka Bhar - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Annalise Galbraith** - Department of Microbiology and Cell Science, Undergraduate Research, Institute of Food and Agricultural Sciences, University of Florida; **Mariola Edelmann** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Melissa Jones** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Background: Human norovirus is one of the most common causes of gastroenteritis. It induces morbidity and mortality by diarrhea and has a massive economic impact resulting in approximately $60 billion each year in healthcare costs and missed worker productivity. Noroviruses are

known to interact with gram-negative enteric bacteria, and this facilitates infection. However, the microbiome- norovirus-host communication link is missing. Noroviruses infect immune cells present in lamina propria, but bacteria themselves are too large to cross the mucosal and epithelial barrier separating gut lumen from lamina propria, making the mechanism by which they enhance infection unclear. We hypothesized that binding of noroviruses to bacteria enhances membrane vesicles (MV) production. MVs readily cross the epithelial barrier and provide a means of communication between the commensal gut flora and the host. Thus, these vesicles have the potential to serve as a mechanism for bacterial enhancement of norovirus infection.

Methods: Attachment assay: Purified murine norovirus (MNV) was incubated with Enterobacter cloacae, Lactobacillus acidophilus and Bacteroides thetaiotaomicron, and grown to produce MVs. Viral attachment was confirmed via qPCR. Isolation of MVs: Clarified media supernatants were ultracentrifuged at varying speeds and 0.2um filtration. Co-purification of norovirus with the MVs was checked. MV quantification and characterization: MV total protein content was measured by microBCA. The number of vesicles were quantified by Nanoparticle tracking analysis. Scanning and transmission electron microscopy was performed to check quality of MV preparation and to determine if virus was attached to the vesicles. Internal MV protein content was evaluated using MS-HPLC. The infectivity of virus bound to MVs was quantified via TCID50 assay.

Results: Incubation of MNV with commensal bacteria resulted in significant increases in production of MVs compared to uninfected controls. MVs produced in the presence of MNV indicated changes in MV protein content which was confirmed by protein analysis. EM analysis determined association of viruses with both bacteria and the MVs, while also revealing specific changes in bacterial surface structural in bacteria bound by virus compared to mock bacteria. The virus bound to MVs were found to cause infection in naive macrophages.

Conclusion: Interactions between MNV and commensal bacteria causes changes in bacterial EV production, size and content and provides insight into possible mechanisms by which bacteria enhance norovirus infection.

119. SEQUENCE CONFIRMATION OF RANAVIRUS INFECTION IN AMPHIBIANS FROM CHAD, AFRICA

Erin K. Box - Southeastern Cooperative Wildlife Disease Study; **Christopher A. Cleveland** - Southeastern Cooperative Wildlife Disease Study; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, University of Florida; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Michael J. Yabsley** - University of Georgia

Ranavirus infection can have varying effects, but can cause significant mortality events and population declines in amphibian populations. Ranaviruses have a broad global range and have been reported from 6 continents, although the single report from Africa (Cameroon) was not sequence confirmed. The single suspect infection of ranavirus in Africa was an incidental finding in Xenopus longipes. Thus, there is a considerable knowledge gap concerning ranaviruses in Africa. We opportunistically obtained frog tissue samples from 160 amphibians representing 5 genera (Hoplobatrachus, Hylarana, Ptychadena, Pyxicephalus, and Xenopus) from Chad, Africa. Samples were tested for ranavirus infection using a real-time quantitative PCR assay targeting the major capsid protein. A total of 26/160 (16%) frogs tested positive including 15/87 (17%) Hoplobatrachus spp., 1/3 (33%) Pyxicephalus spp., 10/58 (17%) Ptychadena spp., 0/9 Xenopus spp., and 0/3 Hylarana spp. Sanger sequencing confirmed all samples were >99% identity to numerous frog virus-3 (FV3) and FV3-like sequences. Additional gene targets (DNA polymerase and the ribonucleotide reductase alpha and beta subunits) were sequenced to assist in the classification of the virus. Sequences of individual gene targets indicate that the ranavirus detected in frogs in Chad is most similar to tiger frog virus (TFV) which is a FV3-like virus previously isolated from diseased amphibians cultured in China and Thailand. Full genome sequencing of one sample indicates that the Chad frog virus is a well-supported sister group to the TFVs previously determined from Asia. This work represents the first molecular confirmation of ranaviruses from Africa, and is a first step in comparing ranavirus phylogeography on a local and global scale.

120. THE EFFECTS OF SIGMA VIRUS INFECTION ON MATE CHOICE IN DROSOPHILA MELANOGASTER

Andrea Gonzalez - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Enrique Gutierrez** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Marta L Wayne** - Department of Biology, College of Liberal Arts and Sciences, University of Florida

It has been suggested that mate preference is an adaptive strategy that helps mitigating the effects of infectious diseases. Preferring healthy individuals to mate reduce the probability of acquiring an infectious disease, ensure delivery and care of the offspring and, boost the offspring fitness because the genes are good or compatible. Yet, experimental evidence supporting these ideas is lacking. Here, we use the fruit fly Drosophila melanogaster and Sigma virus, a natural vertically transmitted rhabdovirus to study the effects of viral infection in the mating preference of this fly species. We first assessed in a female choice scenario if females copulated more with healthy or sick males. Then, we tested if this infected/uninfected male choice was done by cryptic female choice by looking at the progeny counts after a second male encounter. In a choice scenario, we didn't find statistical differences between females mating with infected or uninfected males. We found higher number of progeny when uninfected females mated an infected male for the second time. This study highlights the importance of studying cryptic female choice to study the mechanisms of spread diseases.

121. THE FIRST NUDIVIRUS SEQUENCE FROM AN INVASIVE AMPHIPOD HOST: AN EVOLUTIONARY INSIGHT INTO CRUSTACEAN NUDIVIRUSES

Thomas W. Allain - Department of Fisheries and Aquatic Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Grant D. Stentiford** - Centre for Environment, Fisheries and Aquatic Science, Weymouth, Dorset, DT4 8UB, UK; **David Bass** - Centre for Environment, Fisheries and Aquatic Science, Weymouth, Dorset, DT4 8UB, UK & Department of Life Sciences, Natural History Museum, London, SW7 5BD, UK; **Donald C. Behringer** - Department of Fisheries and Aquatic Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Jamie Bojko** - School of Health and Life Science, Teesside University, Middlesbrough, TS1 3BA, United Kingdom

The Nudiviridae are a group of large double-stranded DNA viruses that infect the gut tissues of invertebrate animals, including insects and crustaceans. Two nudiviruses have been described from decapod crustaceans and here we present the genome of a recently observed nudivirus infecting the nuclei of the hepatopancreatic cells of the invasive amphipod (Peracarida), Dikerogammarus haemobaphes. The genome of the virus is circular and 119,754bp in length, encoding a predicted 106 open reading frames (ORFs). This nudivirus encodes all the conserved nudiviral core genes, excluding the p6.9 gene. Overall, this virus shares 57 gene homologues with other crustacean-infecting nudiviruses. Phylogenetic analysis revealed that this virus branches before the other crustacean-infecting nudiviruses, placing it within the Gammanudivirus genus. Gene synteny across the crustacean-infecting nudiviruses is conserved for Homarus gammarus nudivirus and Penaeus monodon nudivirus; however, three genomic rearrangements appear to break the gene synteny between this novel virus from a freshwater Peracarid host and the other two from marine decapod hosts. We explore the evolutionary history, putative systematics, and significant ORFs of this novel virus.

122. THE GENOMIC SEQUENCE OF LARGEMOUTH BASS VIRUS AND THE EVOLUTIONARY RELATIONSHIP WITHIN THE GENUS RANAVIRUS (FAMILY IRIDOVIRIDAE)

Kuttichantran Subramaniam - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Briana Vigil** - Department of Biological Sciences, California State University; **Joei Quevedo** - Department of Biological Sciences, California State University; **Kristina S. Hamel** - Department of Biological Sciences, California State University; **Maxine J. Bravo** - Department of Biological Sciences, California State University; **Jeanine Abrams McLean -** University of Texas at Austin; **Vsevolod L. Popov** - University of Texas Medical Branch; **Jan Lovy** - New Jersey Division of Fish and Wildlife; **James K Jancovich** - Department of Biological Sciences, California State University; **Thomas Waltzek** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida,

Largemouth bass virus (LMBV) (family Iridoviridae, genus Ranavirus) is associated with lethal disease of North American bass species (Micropterus salmoides; M. floridanus). LMBV was first observed in Lake Weir, FL in 1991, and since that time outbreaks of LMBV have been observed throughout the Midwestern and Southern United States. Major symptoms of LMBV disease include lesions and over-inflation of swim bladders, which alter equilibrium and prevent submergence of infected hosts. The level of susceptibility and the degree of infection differ among outbreaks as some show detrimental symptoms, while others appear unaffected by exposure to LMBV. It is unclear if this variability is due to dissimilarities of immune responses between host populations, or due to the pathogenic diversity among LMBV strains from different geographic regions. Therefore, genomic sequencing of LMBV will allow us to gain a better understanding of this important pathogen of largemouth bass. In addition, having complete genomic sequence information for LMBV will provide insight into the evolutionary relationship among fish iridoviruses and increase our understanding of how ranaviruses infect such a wide variety of hosts. We have sequenced the genome of LMBV using next generation sequencing technology and the assembled LMBV genome has a unique organization. Dot plot comparisons between LMBV and all of the completely sequenced ranavirus genomes show that LMBV is not completely co-linear with any known ranavirus genome. In addition,

phylogenetic analysis using the 26 core iridovirus genes shows that LMBV is a unique taxonomic group within the genus Ranavirus. Together, these data support the hypothesis that last common ancestor for the amphibian-like ranaviruses was a fish virus and that jumps from fish to other cold-blooded vertebrates have occurred during ranavirus evolution. In addition, these data show that the Santee-Cooper ranavirus group forms a unique clade within the genus Ranavirus. As a result, there appears to be a need for taxonomic reorganization within the family Iridoviridae and genus Ranavirus. Therefore, possible changes in ranavirus taxonomy will be discussed.

123. TORQUE TENO SUS VIRUS 1 (TTSUV1) AS A SURROGATE PATHOGEN FOR MODELING FOREIGN ANIMAL DISEASES

Brandon Parker - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Joshuah A. Demetrius** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Raoul K. Boughton** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **James C. Beasley** - Savannah River Ecology Laboratory and Warnell School of Forestry and Natural Resources, University of Georgia; **James D. Austin** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Ryan S. Miller -** USDA Animal Health Plant Inspection Service, Center for Epidemiology and Animal Health, Natural Resource Research Center; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Understanding how foreign animal diseases like African Swine Fever and Swine Flu spread is fundamental for preventing epidemics; however, studying the pathogens that cause these diseases can be impractical or costly. Surrogate pathogens can instead be used to safely model how these pathogens are transmitted within and among populations. An ideal surrogate pathogen to study transmission would have 1.) high prevalence 2.) low virulence and 3.) similar transmission routes and tissue dissemination to the pathogen it is modeling. Torque teno sus virus (TTSuV) is a genus of viruses in the Anelloviridae family that infect swine and previous studies suggest it may be a good surrogate. To verify Torque

teno sus virus 1 (TTSuV1) meets the criteria for an ideal surrogate pathogen we collected biosamples from a total of 167 wild pigs at two sites, Archbold's Buck Island Ranch (ABIR) and Savannah River Site (SRS). Using viral DNA, we estimated viral genetic identity across time and space. We also calculated prevalence across multiple tissue types and estimated ages in wild pigs. TTSuV1 was found in 87 pigs and overall prevalence of the virus was 50.9% with no significant difference between trapping locations. Whole blood had the highest prevalence at 50.2% and genital, oral, and nasal swab samples had lower prevalence at 0-7%. Viral sequences were aligned using a 678 base pair amplicon to establish genetic diversity of TTSuV1. Viral genetic identities correlated with host social groups and showed that virus in wild pigs in the same sounder was more similar than among sounders. This suggests the importance of direct transmission of TTSuV1 within social groups. Low virulence was also implied from the high prevalence of TTSuV1 across all age groups. Our results showed that TTSuV1 fulfills the criteria of a surrogate pathogen to model the transmission of foreign animal diseases.

124. A MACROPHAGE-BASED SCREEN YIELDS HOST-TARGETING MODULATORS OF BACTERIAL UPTAKE

Autumn Dove - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Alyssa Walker** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Gustavo Maegawa** - Department of Pediatrics, University of Florida; **Daniel Czyz** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

The stagnation of antibiotic development and the dramatic rise in antibiotic resistance has created a concerning gap in the availability of effective antimicrobials. Antibiotic resistance claims nearly 50,000 lives in the United States each year, and it is estimated that by 2050 this number will increase tenfold. There is an urgent need to develop alternative therapies against antibiotic-resistant infections. Our work concentrates on repurposing current drugs for their ability to inhibit infection by targeting the host. Drug repurposing can potentially lower the cost and speed up the development of new therapeutics. Additionally, targeting the host, but not the pathogen, will circumvent antibiotic resistance. Host-targeting compounds could be used in combination with current antibiotics. We used a novel method of quantifying intracellular bacteria to explore modulation of bacterial entry into cells as a potential host-targeting mechanism against antibiotic-resistant bacteria. For extracellular bacteria, the enhancement of uptake should allow for an increased clearing of the infection, while inhibiting bacterial uptake should attenuate infections by intracellular bacteria. To this end, we used a library of 2,400 approved drugs to search for modulators of Escherichia coli uptake by RAW 264.7 macrophages. We identified 31 compounds; among these three enhanced and 28 inhibited bacterial uptake at no cytotoxicity to bacteria or host cells. We demonstrated that one of the uptake inhibitors attenuated infection by Salmonella, Pseudomonas, Klebsiella, and Acinetobacter species. We are currently exploring the mechanism of the inhibition of uptake. This screen highlights the

feasibility of using host-targeting compounds as immunomodulators in the treatment of antibiotic-resistant infections.

125. A STUDY OF GUT MICROBIOTA DYNAMICS IN PARKINSONIAN MICE

Emily Klann - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Massimiliano Tagliamonte** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Maria Ukhanova** - Emerging Pathogens Institute, University of Florida; **Vinata Vedam-Mai** - Department of Neurology, Center for Movement Disorders and Neurorestoration, College of Medicine, University of Florida; **Volker Mai** - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Background: Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by both motor and non-motor symptoms, resulting from the death of dopaminergic neurons due to an accumulation of misfolded alpha-synuclein. This presumably initiates a cascade of immune events which result in persistent neuroinflammation. Given that there is impaired peripheral immunity in PD, there exists an avenue for therapeutic interventions such as adoptive cellular therapy (ACT). Here we used a preclinical model of PD (M83 mice) to test the effects of ACT on gut microbiota composition, considering the strong connection between the intestinal milieu and CNS.

Methods: A total of 17 M83 mice (transgenic mice expressing the human form of familial A53T mutant alpha-synuclein) were used in this study. Mice in the treatment arm (N=10) were injected with adoptive cellular therapy vaccine (ACT), and control mice (N=7) were injected with saline at 8 weeks of age. All mice received a peripheral, intramuscular injection of alpha-synuclein fibrils one week later (9 weeks of age; baseline). Fecal pellets were collected from each mouse at three time points (baseline, 6 weeks and 12 weeks). 16S bacterial DNA from each stool sample was extracted, sequenced, and analyzed using QIIME2 and RStudio.

Results: Overall, mice treated with ACT outsurvived the control mice and exhibited fewer motor deficiencies. Microbiota analyses revealed differences in the relative abundance of certain phyla over time between the two groups. Interestingly, we observed statistically significant

increases in the relative abundance of Actinobacteria following ACT in the treatment group (p=0.021). Further, Actinobacteria was significantly more abundant in the treatment group compared to controls at the timepoint following injection (p=0.022). We found no significant differences in alpha diversity (Shannon Diversity) between treatment and control groups at any timepoint. However, the alpha diversity measures for the treatment group demonstrated a decreasing trend over time that was absent in controls. Unweighted UniFrac measures of phylogenetic distance between samples demonstrated distinct clustering in all samples at baseline as well as between treatment and control mice at times 1 and 2 post-baseline (p=0.002).

Conclusion: Mice that received ACT demonstrated improved survival and behavioral measures as well as significant differences in the composition of the gut microbiome compared to controls. These results suggest an underlying relationship between the gut-brain-axis and PD pathology, with respect to ACT, which warrants further research.

126. BUTYRATE SUPPRESSES BACTERIA-INDUCED PROTEIN AGGREGATION IN C. ELEGANS MODELS OF PROTEIN CONFORMATIONAL DISEASES

Alyssa Walker - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Emily Donahue** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Alfonso Vaziriyan-Sani** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Benjamin Hoffman** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Autumn Dove** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Daniel Czyz** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

Recent discoveries demonstrate that bacteria contribute to the pathogenesis of protein conformational diseases. However, the identities of bacteria that influence protein folding in their host and the underlying mechanisms by which this influence is executed remain largely unknown. Here, we describe our efforts to decipher the role of pathogenic bacteria that reside in the human gut on host proteostasis using the C. elegans

intestine. We found that opportunistic pathogens often associated with gastroenteritis—including members of the Enterobacteriaceae family and other species such as Listeria, Vibrio, Pseudomonas, and Acinetobacter—enhanced protein aggregation upon colonization of the intestine. Interestingly, these bacteria not only disrupt the protein folding environment in the host intestine, but also in neurons and muscle tissue. Furthermore, we observed that colonization of the C. elegans intestine with proteostasis disrupting bacteria enhanced polyglutamine aggregation in the F1, but not the F2 generation. Taken together, these data suggest that bacteria may release “signals” that affect distal tissues, including the gonad. Many commensal bacteria produce short-chain fatty acids; among these molecules, butyrate was recently shown to mitigate the severity of protein conformational diseases. We found that butyrate enhanced bacteria-mediated protein aggregation at low physiological concentrations but suppressed bacteria-induced aggregation at high therapeutic concentrations. To examine the effect of butyrate-producing bacteria on host proteostasis, we colonized the C. elegans intestine with bacteria engineered to overproduce butyrate. The resulting increase in endogenous butyrate enhanced proteostasis in the intestine and other tissues, as assessed by a decrease in protein aggregation in the host. Collectively, our results demonstrate that pathogenic enteric bacteria disrupt host proteostasis and enhance aggregation of metastable proteins. These findings suggest that dysbiosis between commensal butyrate-producing bacteria and pathogenic enteric species may have a detrimental effect on host proteostasis.

127. CHARACTERIZATION, ABUNDANCE, AND DIVERSITY OF ANTIMICROBIAL RESISTANT PATHOGENS IN WASTEWATER TREATMENT TRAINS

Karen Sem - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Katherine Deliz-Quiñones** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **John Bowden** - Department of Physiological Sciences, College of Veterinary Medicine, University of Florida

Wastewater treatment facilities (WWTFs) utilize secondary and tertiary treatments that are designed to remove chemical and biological pollutants, but the harsh conditions that they create may select for antimicrobial resistance (AMR). The spread of AMR from reclaimed water and sludge into receiving environmental microbiomes can lead to the propagation and retention of AMR-conferring genes throughout the natural environment, reducing the potency of antimicrobials which these genes confer resistance against and threatening the efficacy of their common uses, such as in food production and the prevention and treatment of microbial infections. This presentation examines the composition of antimicrobial resistant pathogens throughout various WWTF treatment trains. Samples were taken from each of the treatment stages in a municipal WWTF and a university WWTF in north central Florida. The identity and relative abundance of pathogenic species present in each sample were found using biochemical tests performed on colony dilutions and DNA extraction and sequencing. Through observing changes in the occurrence and abundance of pathogenic species in each stage of the facility, the effect of the corresponding secondary or tertiary treatment on AMR selection can be determined. This information can contribute to the development of more effective treatments and management strategies that target the removal of antimicrobial resistant pathogens from wastewater.

128. COMPARING DISSECTION METHODS FOR DETECTING DOG HEARTWORM IN AEDES AEGYPTI

Nicole O. Abruzzo - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

Dirofilaria immitis (Leidy), a domestic dog parasite, is vectored by mosquitoes. Due to the veterinary importance of D. immitis, efficient identification of its mosquito vectors is necessary to identify target species for vector management. Current mosquito screening methods involve separating the abdomen, which contains the non-infective forms of the parasite, from the head and thorax, which contain the infective third larval stage parasites. This study compared the traditional dissection method with an intact mosquito screening protocol that did not involve dissection. Aedes aegypti infected with one of two D. immitis strains were dissected at a timepoint when they would harbor either infective or non-infective nematode lifestages. Uninfected Ae. aegypti also were dissected. DNA was extracted from both dissected (head/thorax and abdomen) and intact mosquitoes of each type and samples were screened for D. immitis with qPCR. Of the laboratory-reared, Missouri strain (MO), 15 day post-bloodfed samples, D. immitis was detected in all mosquito head/thorax samples and 30% of the intact mosquito samples. Ninety percent of the 1-hour post-bloodfed MO intact mosquito samples and 30% of the MO head/thorax samples had detectable D. immitis. Mosquitoes infected with the JYD D. immitis strain and mosquitoes dissected at additional post-bloodfeeding time points are currently being processed. This study seeks to determine the most efficient means for screening potentially infective D. immitis mosquito vectors to improve accuracy of surveillance efforts across multiple strains of the parasite.

129. CONSISTENT INDIVIDUAL DIFFERENCES IN BEHAVIOR IN A FACULTATIVELY PARASITIC MITE

Emily Durkin - Department of Biology, University of Florida; **Allison Roth** - Department of Biology, University of Florida; **Carl N. Keiser** - Department of Biology, University of Florida

Host attachment is a necessary behavior in the evolution of parasitism. It is hypothesized that populations pass through a stage of facultative parasitism before the evolution of obligate parasitism. Previous research has revealed the impact of extrinsic factors on the expression of parasitic tendencies, but to our knowledge, the role of individual behavioral variation driving parasitic tendencies remains to be tested. Here, we used repeated behavioral assays to quantify attachment propensity and activity level of individual Macrocheles muscaedomesticae, facultatively parasitic mites of flies, from two different populations. Mites from both populations exhibited repeatability in attachment propensity and mites from one population exhibited repeatability in activity level. We did not find a relationship between an individual's activity level and attachment propensity in mites from either population. Our data suggest that facultative parasitism may not simply describe a phenotypically plastic strategy that responds to environmental cues, but perhaps that individual differences in parasitic tendencies may appear like facultative parasitism at the population or species level.

130. EFFECT OF TEMPERATURE AND LEAF WETNESS DURATION ON AN EMERGING FOLIAR DISEASE OF AN INVASIVE GRASS

Ashish Adhikari - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Amy Kendig** - Department of Agronomy, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **S. Luke Flory** - Department of Agronomy, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Philip F. Harmon** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Erica Goss** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Microstegium vimineum (Trin.) A. Camus, common name stilt grass, is a highly invasive annual c4 grass dominating the forest ecology of the eastern USA. Stricker et al. (2016) reported a Bipolaris species, designated B. megaspora but more recently identified as Drechslera gigantea, as a major fungal pathogen of Microstegium. The major symptoms include eyespot lesions on leaves with grey white center and brownish margin. However, the disease is not present throughout the invaded range of Microstegium. Our objective was to determine the temperature and moisture requirement for disease development on invasive Microstegium to better understand the epidemiology and distribution of this fungal pathogen. We conducted experiments in controlled environments to measure the effect of temperature regimes and leaf wetness duration on infection by D. gigantea and disease severity on Microstegium. Six weeks old Microstegium seedlings were inoculated with 2*104 conidial suspension of D. gigantea and bagged to maintained 100 % leaf moisture and wetness. Inoculated plants were placed into three different incubators set at three different temperature regimes: Ta, Tb and Tc representing the mean average day and night temperature of late July, August and mid-September at our southern Indiana field site, where we have been conducting complementary field experiments. On days 0, 1, 3, 5, and 7 days, four plants from each incubator were randomly selected, bags were opened, wet leaves were dried, and plants returned to the incubator. Mock inoculated controls were given the same treatment. At all three temperature regimes, one day of leaf wetness was enough for

infection. Disease severity increased with increasing leaf wetness days. Disease severity was significant different among temperature regimes and moisture durations. Disease severity was higher for the Tb (August) regime compared to Ta and Tc (July and September, respectively). These results highlight the importance and requirement of temperature and leaf wetness for infection and disease progression and are a first step towards explaining the patterns of disease observed in Microstegium populations.

131. EXPLORING DENGUE VIRUS THROUGH A BLENDED FORMAT: COMBINING HANDS-ON LABORATORIES WITH THE WISE PLATFORM IN PRECOLLEGE CLASSROOMS

Julie Bokor - Center for Precollegiate Education and Training, University of Florida; **Danielle Ouellette** - Center for Precollegiate Education and Training, University of Florida

Situated in the Dengue outbreak of 2009 in Key West, Florida, this curriculum unit follows the disease progression and eventual diagnosis of the first case of the outbreak. Each lesson begins with a passage from her case report, challenging the students to track the symptoms, suggest tests, and actually take on the role of medical doctor and clinical laboratory technician. Students perform an ELISA and gel electrophoresis and discuss the results. Through the lesson sequence, students discover the body's immune response and why different tests are used at different stages of the disease progression. Difficulty in developing an effective vaccine is also discussed and students debate the ethical dilemmas surrounding containing and preventing outbreaks of dengue virus. These original lessons as well as extension lessons have been developed in the Web-based Inquiry Science Environment (WISE) on-line platform to allow further dissemination and adaptability to different classroom contexts. In the on-line platform, students investigate the mosquito life cycle and consider how to halt the spread of dengue, including the use of genetically modified mosquitoes as well as assume the role of field epidemiologists in Old Town, Key West, to determine what serotypes of dengue are circulating in the community. Initial pilot testing with teachers and precollege students indicates high levels of engagement and learning through the use of the blended format.

132. EXPLORING EMERGING PATHOGENS CONTENT IN PRECOLLEGE CLASSROOMS

Julie Bokor - Center for Precollegiate Education and Training, University of Florida; **Mary Jo Koroly** - Department of Biochemistry and Molecular Biology, Center for Precollegiate Education and Training, College of Medicine, University of Florida; **Cynthia Nazario-Leary** - Center for Precollegiate Education and Training, University of Florida

Now entering its fourth year, CATALySES (Collaborating to Advance the Teaching and Learning of Science Educators and Students): Emerging Pathogens is a professional development program for secondary STEM teachers developed and implemented by the UF Center for Precollegiate Education and Training (CPET) in collaboration with the Emerging Pathogens Institute. CATALySES is funded by an NIH SEPA grant; teachers participating in the program do not incur any costs. The content of this two-week residential program is focused on infectious diseases and translational research, and is delivered through a combination of hands-on laboratory experiences, workshops, lectures, tours, and discussion groups. The objectives of CATALySES include improving teachers' design expertise, lesson planning, and science identity, leading to improvement of their students' content knowledge, attitudes, and science identity. These students will be better prepared to explore the continuum of paths to the science and health-related workforce. During the two-week summer institute, teachers work with UF science and education researchers to develop lessons and laboratory exercises, translating their CATALySES experiences into classroom action. Each teacher creates a "Research Action Proposal" in which they choose an intervention to implement in their classroom. They decide how they will collect, analyze, and interpret data from their intervention. These action proposals include specific connections to content from the CATALySES institute. Continued support from the CPET CATALySES research team encourages the teachers' personal enrichment and professional advancement in biotechnology education. CPET provides a variety of resources to CATALySES teachers including a stipend to each teacher attending the summer program, free use of an equipment locker program, and an option to participate in CPET'S Special Explorations for Teachers and Students (SETS) program at no cost to them.

133. FOOD LEVEL INFLUENCE ON SPINOSAD EFFICACY IN CONTAINER-DEVELOPING MOSQUITOES

Andrew Branch - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Blair Siegfried** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

Container-developing mosquitoes, such as Aedes aegypti and Ae. albopictus, commonly reside in urban environments and are vectors of pathogens that impact human health. Management of these sites is vital in reducing adult populations, thereby potentially reducing pathogen transmission. Spinosad is a highly promising, naturally-derived insecticide that produces neurotoxic effects when ingested by mosquito larvae. Due to the variability in the habitats in which Aedes larvae reside, tests were conducted to determine if the food level within containers influenced the efficacy of spinosad. Each test consisted of four spinosad treatments, reflecting Ae. aegypti and Ae. albopictus LC50, LC80, LC90, and an untreated control, each receiving one of four of food levels (0.00, 0.01, 0.02, and 0.04 g) added to 50 mL of water. Food level did not have a significant effect on larval mortality in the LC50 spinosad treatment in either species tested. Significantly higher mortality was observed in treatments receiving the 0.01 g food treatment than the 0.04 g level at LC90 spinosad concentration in both species tested. These results suggest the nutrient levels in the larval environment may affect the efficacy of spinosad treatments. Integrated pest management programs utilizing spinosad as a larval control measure may need to factor in food levels within the larval environment to ensure the desired level of population control is achieved.

134. GUT MICROBIOTA-DERIVED HYDROGEN SULFIDE IS REDUCED IN SPONTANEOUSLY HYPERTENSIVE RATS

Basak Donertas - Department of Physiological Sciences, College of Veterinary Medicine, University of Florida; **Wendi L. Malphurs** - Department of Physiological Sciences, College of Veterinary Medicine, University of Florida; **David M. Baekey** - Department of Physiological Sciences, College of Veterinary Medicine, University of Florida; **David Julian** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Jasenka Zubcevic** - Department of Physiological Sciences, College of Veterinary Medicine, University of Florida

Introduction: Gut bacteria play a significant role in host homeostasis, and gut dysbiosis has been associated with many conditions including hypertension (HTN). Endogenous hydrogen sulfide (H2S) is an important freely-diffusing molecule that can modulate neural, cardiovascular and immune system activity, and circulating levels of H2S are reduced in animals and humans with HTN. While most research on the role of H2S in HTN has focused on H_2S produced by host tissues, H2S produced by gut bacteria may also enter the host circulation. We investigated whether the spontaneously hypertensive rat (SHR), an established model of HTN, has a disruption in gut bacteria-derived H2S production.

Methods: Bacterial DNA from fecal samples of adult male normotensive Wistar–Kyoto (WKY) and SHR was isolated for 16s bacterial genomic sequencing. WKY and SHR fecal and plasma samples were analyzed for H2S levels using modified methylene blue assay. Blood pressures were determined in all rats at several time points using a tail cuff. Data are expressed as mean±SEM, with $P<0.05$ considered statistically significant.

Results: We found a significant reduction in the abundance of two H2S-producing gut bacteria in the SHR compared to WKY: Enterobacteriaceae (1.423e-005±1.056e-005 vs. 0.0002329±7.853e-005, n=6, $P<0.05$), and Clostridiaceae (0.01134±0.00431 vs. 0.06408±0.01416, n=6, $P<0.01$). This decrease in the H2S-producing gut bacteria was also associated with a significant reduction in fecal H2S levels (SHR: 0±0.01703 AU vs. WKY: 0.094±0.03385 AU, n=5; $P<0.05$) and lower plasma H2S levels (SHR: 0.3265±0.07817 AU vs. WKY: 0.6850±0.1790 AU, n=2) in the SHR compared to the WKY.

Conclusion: These results suggest that diminished gut bacterial production of H2S may contribute to the reduced H2S observed in host circulation in established HTN.

135. ISOLATION OF BACTERIOPHAGES IN FLORIDA OYSTERS INFECTING CLINICAL AND ENVIRONMENTAL VIBRIO PARAHAEMOLYTICUS ISOLATES

Siman Liu - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **David Gonzales** - Department of Microbiology and Cell Science, University of Florida; **Paul Gulig** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida; **Naim Montazeri** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: Foodborne infections with Vibrio parahaemolyticus are commonly associated with the consumption of raw oysters grown in contaminated seawater. Metagenomic analyses showed that V. parahaemolyticus likely acquire virulence factors following infection with bacteriophages. Despite a high abundance of Vibrio spp. in Florida's coastal areas, little is known about the prevalence of their bacteriophages and how their interaction leads to the emergence of virulent strains.

Purpose: To study V. parahaemolyticus bacteriophages isolated from Florida oysters.

Methods: Five environmental and five clinical isolates of V. parahaemolyticus were utilized as host cells to enrich bacteriophages from wild-caught oysters (Cedar Key, FL). Plaque assay performance was examined for bacteriophage isolation utilizing top agar either supplemented with 0.0 to 8.0 mM divalent salts (MgCl2 and CaCl2) or prepared with 2.4% salinity seawater. Following three rounds of plaques purification, host specificity was carried out using spot plate assay. The presence of prophage in V. parahaemolyticus host strains was determined using mitomycin C treatment.

Results: Except for a marginally higher plaque yielded with 5 mM divalent salts in top agar, no noticeable difference was observed in plaque size and turbidity among different conditions. To resemble a marine environment, top agar prepared with seawater was utilized for plaque isolation. Overall, six bacteriophages were isolated and resulted in ~0.5

mm in diameter, clear and turbid plaques. Two out of ten strains of V. parahaemolyticus, i.e. VP FDA R2 (tdh-, trh+) and VP FDA R75 (tdh+, trh+), isolated from oysters, did not have prophage and showed a higher rate of bacteriophage propagation yielding to 106 PFU/mL. Bacteriophages 9A and 10A resulted in the widest host range by infecting eight and seven host strains.

Significance: The findings from this study serve as our first attempt to further elucidate the bacteriophage-vibrio interaction and apply it for developing novel risk-assessment tools.

136. MITIGATION OF EQUINE RECURRENT UVEITIS THROUGH TOPICAL SUPPRESSOR OF CYTOKINE SIGNALING-1 (SOCS1) MIMETIC PEPTIDE: OPEN LABEL SAFETY AND EFFICACY PILOT STUDY

Timothy Polk - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Jatin Sharma** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Sarah Bae** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Olivia Barr** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Amari Jones** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Holly Kitchen** - Department of Small Animal Clinical Sciences, Institute of Food and Agricultural Sciences, College of Veterinary Medicine, University of Florida; **Michelle Wilhelmy** - Department of Small Animal Clinical Sciences, Institute of Food and Agricultural Sciences, College of Veterinary Medicine, University of Florida; **Katherine Devin** - Department of Small Animal Clinical Sciences, Institute of Food and Agricultural Sciences, College of Veterinary Medicine, University of Florida; **Joseph Larkin III** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Caryn Plummer** - Department of Small Animal Clinical Sciences, Institute of Food and Agricultural Sciences, College of Veterinary Medicine, University of Florida,

Recurrent uveitis is a relapsing/remitting disease of often increasing severity that accounts for more than 10% of severe visual handicaps diagnosed in human patients in the United States. Notably, equine recurrent uveitis (ERU) is the only spontaneous model of human recurrent uveitis, occurs in 10% of all horses, is a leading cause of equine blindness, and often results in horse euthanasia. Uveitis, which specifically refers to the inflammation of the uveal tract, is a collection of sight-threating intraocular diseases that are driven by aberrant inflammation from the immune system. These decreases or losses of sight occur despite standard of care treatments that largely include the use of topical steroids. Even when effective, prolonged steroid use is generally accompanied by many significant negative side effects. There is, therefore, a critical need for the development of novel therapeutic strategies to treat equine and human recurrent uveitis. Notably, the body naturally generates a family of intracellular proteins, known as suppressors of cytokine signaling (SOCS), which prevent excessive inflammatory responses. Significantly, we have shown that topical application of an eye drop, containing a cell-penetrating peptide that mimics suppressor of cytokine signaling-1 (SOCS1-KIR), could mitigate experimental autoimmune uveitis in mice. We have conducted bioinformatic analysis studies that demonstrate that the SOCS1-KIR domain is highly conserved in mice, horses, and humans. In order to evaluate the ability to translate our preclinical findings we conducted a first in equine, open label study in which we evaluated the safety and efficacy of topical SOCS1-KIR peptide administration in equine recurrent uveitis (ERU) patients. We found that topical SOCS1-KIR administration was well tolerated in equine patients. Moreover, topical administration of SOCS1-KIR peptide twice a day was associated with reductions in ocular discomfort, hyperemia, and aqueous flare. Finally, biochemical analysis revealed that SOCS1-KIR peptide could be enriched from a complex protein mixture using ammonium sulfate precipitation, providing a path moving forward for optimization of ocular peptide dosing. Together, these results provide justification for conducting a large multicenter trial to evaluate an optimized SOCS1-KIR treatment in horses, and further exploration of SOCS1-KIR in the treatment of human ocular diseases.

137. MODE OF ACTION OF THE PLANT ALKALOID LIRIODENINE ON MOSQUITO AND OTHER INSECT NERVOUS SYSTEMS

Quentin Coquerel - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Fabien Demares** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Anne-Marie Le Rey** - Département de Pharmacologie, Université d'Angers, France; **Dimitri Breard** - Département de Pharmacologie, Université d'Angers, France; **Pascal Richomme** - Département de Pharmacologie, Université d'Angers, France; **Christian Legros -** Département de Pharmacologie, Université d'Angers, France; **Jeffrey Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida

Alkaloids are secondary metabolites synthesized by living organisms and act in a wide variety of functions: antimalarial (e.g. quinine), analgesic (e.g. morphine) anti-arrhythmic (e.g. quinidine), and insecticidal (e.g. nicotine). Liriodenine is an oxoaporphine alkaloid isolated from various plant families, such as Annonaceae or Magnoliaceae. It has been shown to have different effects on mammals, fungi, and bacteria, but has never been assessed for insecticidal activity. We tested liriodenine at different levels: for toxicity to Anopheles gambiae adult mosquitoes via direct topical application; on Drosophila melanogaster central nervous system (DmCNS) by recording the firing activity of the ventral nerve cord; on a Musca domestica larva peripheral neuromuscular preparation, on Periplaneta americana dissociated neurons, and on Anopheles AgKv2.1 potassium channels expressed in HEK293 cells. From adult mosquitoes, we observed that liriodenine was lethal after 24 hours, and both dimethyl sulfoxide (DMSO) and piperonyl butoxide (PBO) synergize its action. Also, liriodenine targeted both central and peripheral nervous systems in flies, suggesting neurotoxicity. Since liriodenine's structure is close to the one of bicuculline, a known GABA antagonist acting in mammals but not in insects, we first investigated GABA neurotransmission. The inhibitory action of GABA on the firing activity of DmCNS was reversed by liriodenine (IC50 = 42 µM), and this observation was corroborated by the block of GABA-induced currents on patch-clamped cockroach isolated neurons (IC50 ≈ 1 µM). As a control, we also tested bicuculline, which is known to be inactive on insect GABA receptors and did not block GABA-induced currents. On the Musca neuromuscular junction, liriodenine

(starting at 10 µM) induced a significantly faster repolarization of excitatory post-synaptic potentials. This result suggested that liriodenine affected another target, different from GABA, possibly potassium currents as they are involved in action potential generation. This possibility was confirmed by the block of delayed-rectifier potassium channels AgKv2.1 by liriodenine (IC50 = 2.1 µM). This is the first study assessing the toxicology of liriodenine on insects. We report two distinct targets, central GABA receptors and potassium channels, both affected by liriodenine with similar affinities.

138. OZONE GENERATION AND DISTRIBUTION BY DIELECTRIC BARRIER DISCHARGE PLASMA REACTORS FOR ENHANCED DISINFECTION

Bhaswati Choudhury - Department of Mechanical and Aerospace Engineering, Emerging Pathogens Institute, College of Engineering, University of Florida; **Emma Noelle Mastro** - College of Engineering, University of Florida; **Paulina Wiktoria Kapuscinska** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Judith Johnson** - Department of Pathology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Subrata Roy** - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida

Enhanced distribution of atmospheric Surface Dielectric Barrier Discharge (SDBD) plasma generated ozone in air for effective disinfection is examined. This is achieved through experimental investigation of the effect of better ozone distribution on disinfection and ozone distribution produced by SDBD plasma reactors. This study provides an alternative technique for disinfection of cleanroom facilities, objects and surfaces. Advantages associated with atmospheric SDBD plasma disinfection like low temperatures, no organic-residuals and design flexibility make it a potential alternative to conventional methods like thermal, chemical and radiation treatments. In this study, the effect of better ozone distribution on disinfection is first examined using an external fan for distributing ozone generated by a comb SDBD reactor. Spore forming bacterial species, Bacillus subtilis, commonly used in evaluating disinfection methods, was used as the test organism in these experiments. Significant reduction (~78%) of bacterial concentrations in the inoculated volume of air was observed using the comb shaped SDBD plasma reactor. Increased

reduction (~98%) was observed when the same reactor was used in conjunction with an external fan for better ozone distribution. Considering this, a new DBD fan configuration can be used instead of the external fan to better distribute ozone leading to lower ozone requirements. For this purpose, comparative study of ozone distribution in a chamber resulting from the flow produced by two SDBD reactor configurations: (a)Comb reactor and (b)the recently developed Fan reactor, is performed. The ozone distribution data showed that the Fan SDBD reactor produced a more uniform distribution in the enclosed volume when compared to the Comb reactor. This suggests that the Fan reactor would result in enhanced disinfection by requiring less overall ozone concentrations due to better distribution and mixing. Disinfection observed in inoculated (Escherichia Coli) agar plates placed at the center of four walls of a chamber resulting from ozone distribution by the Comb and Fan reactor is also presented to support this idea. Thus, this research indicates that better ozone distribution results in increased disinfection. Considering this, ozone generation and distribution capability of SDBD reactors were examined along with resulting disinfection at the center of four chamber walls, by performing a comparative study between a conventional Comb reactor and the recently developed Fan reactor. The results show that the recently developed Fan reactor has the potential of achieving enhanced disinfection compared to the comb reactor and possibly other reactor configurations.

139. REDUCTION IN HAZARDOUS DRINKING REPORTED AMONG WOMEN WITH HIV ENROLLED IN THE WHAT-IF? STUDY

Shantrel Canidate - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Christa Cook** - University of Central Florida; **Nicole Ennis** - Florida State University; **Giselle Carnaby** - University of Central Florida; **Zhi Zhou** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Robert Cook** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida

Background: Considerable efforts have been made in the field of public health to reduce alcohol consumption and improve overall health outcomes for people living with HIV. Among women with HIV, an estimated 6-54% engage in hazardous drinking, a behavior that can lead to additional personal and public health consequences. While treatment seeking among women with an alcohol use disorder (AUD) is relatively low compared to men, women have reported better overall treatment outcomes. The purpose of this study is to better understand why some women with HIV who enrolled in a clinical trial were more successful than others in reducing or quitting drinking.

Methods: Convenience and theoretical sampling was used to recruit women with HIV and hazardous drinking to complete qualitative interviews which asked questions pertaining to the multiple determinants of drinking behavior. These women had previously completed the WHAT-IF? (Will Having Alcohol Therapy Improve my Functioning?) clinical trial and had consented to be contacted in the future for study related purposes.

Findings: A total of 20 women with HIV completed the qualitative interviews. Notable reasons for reducing/quit drinking included: the opportunities for self-reflection and change through completing the study assessment, fear of adverse events associated with drinking and taking the study medication, and positive interaction with the research study staff who provided encouragement and support during enrollment in the study.

Conclusion: There is a critical need to understand why women with HIV drink as well as the salient factors that led to drinking behavior change in

women with HIV with hazardous drinking. Despite relapse rates up to 70%, finding suggest participation in a clinical trial to reduce drinking among women with HIV could lead to improved health outcomes not necessarily dependence on medication but other aspects of the clinical trial (e.g. completing study assessments, interactions with the research staff).

140. RESISTANCE EXPRESSION OF PERMETHRIN AND FIPRONIL IN MULTIPLE RHIPICEPHALUS SANGUINEUS POPULATIONS

Yuexun Tian - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Phillip Kaufman** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Cynthia Lord** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida

The brown dog tick, Rhipicephalus sanguineus Latreille, is a canine ectoparasite, which can establish populations in dog kennels and residences. Residential infestations may start with a few engorged females and rapidly increase to high infestation levels with multiple tick stages appearing at the same time. Detection of ticks by home owners may be difficult during the early stages due to low tick numbers, causing a delay in the onset of tick control. Brown dog tick management involves multiple chemical acaricides that are used both on-host and off-host. Permethrin and fipronil are two common acaricides that are used to manage brown dog tick infestations. Permethrin acts on sodium channel and can be used both on and off host, while fipronil blocks chloride ions flow by affecting GABA-gated chloride channel and is used only on host. However, home owners have reported the failure of preventing or managing brown dog ticks using permethrin and fipronil. Previous studies have demonstrated brown dog tick populations from Florida and Texas have high resistance (resistance ratio greater than 10) to permethrin and tolerance (resistance ratio between 1 and 10) to fipronil. In this study, acaricide resistance expression of three brown dog tick strains, a laboratory strain (previously shown susceptible to both chemicals) and two field-collected strains, one from Imperial County, California and one from Port St. Lucie, Florida, were screened for susceptibility to permethrin and fipronil. LD50, LC90, and resistance ratios were calculated

to allow comparison between California and Port St. Lucie strains. Our results indicated that the laboratory strain is susceptible to both chemicals, the Port St. Lucie strain has high resistance to permethrin and expressed tolerance to fipronil, and the California strain exhibits lower resistance to permethrin, with fipronil resistance evaluation ongoing. The data obtained from this study will be used to parameterize a brown dog tick developmental model that will improve our understanding of brown dog tick phenology, population dynamics and control.

141. SOCS1 MIMETIC ATTENUATES ANTI-NUCLEIC ACID IGG IN A LUPUS MODEL

Jatin Sharma - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Systemic Lupus Erythematosus (SLE) is an autoimmune disease with a perplexed etiology. The disease mostly affects women and has a prevalence rate of approximately 0.15% worldwide. There is an unmet medical need for SLE patients. The disease is marked by dysregulated immune processes that include the production of anti-self, anti-nucleic acid antibodies (ANA) that drive disease in multiple organs including skin, kidneys, heart, and joints. The immune system, including antibody production, is dependent upon cellular communication through molecules called cytokines. Cytokines generally activate the responsive cells using intracellular proteins called Janus kinases (JAKs), making them an important target of immune regulation. As such, cytokine signaling is regulated by Suppressor of Cytokine Signaling-1 (SOCS1) an endogenous protein that limits Janus kinase activity. Importantly, SOCS1 deficiency and SNPs have been linked to several autoimmune diseases, including SLE. Notably, we have previously shown that peritoneal and topical administration of SOCS1-KIR, a peptide mimic of SOCS1, was effective in preclinical models of multiple sclerosis and uveitis, respectively In our current study, we tested if the daily administration SOCS1 KIR, a SOCS1 mimetic peptide, for 6 weeks could prevent the development of lupus-like pathology that spontaneously occurs in MRL/lpr mice. We found that the peptide treatment was non-toxic, attenuated skin lesions, lymphadenopathy, and serum ANA. We also saw reduction in total T follicular cells (PD1+CXCR5+), germinal center B cells (CD19+ GL7+), and

CD80+ plasma cells (CD19- CD138+). Since aberrant germinal center B cells and plasma cell activation are implicated in many autoimmune diseases like SLE and rheumatoid arthritis, we believe the peptide mimetic may have therapeutic potential.

142. UF CARPENTRIES CLUB

Eve Bohnett - Department of Geography, University of Florida; **Minghao Gong** - Department of Statistics, University of Florida; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Elise Morrison** - Department of Geological Sciences, University of Florida; **Jenicca Poongavanan** - Interdisciplinary Ecology at University of Florida; **Brian Jay Stucky -** Florida Natural Museum of History, University of Florida; **Chaudhary Vratika** - Wildlife Ecology and Conservation University of Florida

The UF Carpentries Club is a group of independent members of the UF community dedicated to providing structured pathways for our colleagues to learn informatics skills outside of the traditional university curriculum. The Club is open to any member of the UF community including staff, faculty, and students, paid, and volunteer. In addition to our core informatics goal, we seek to bring the Carpentries' teaching methods to instructors and to develop a research community dedicated to open and reproducible science. Our main activity is teaching Software and Data Carpentry workshops. Our instructors, workshop members, and board all serve on a volunteer basis. Most workshops use lesson materials from the Carpentries. These are designed to provide novice users with a foundation to continue learning on their own, with their peers, or more formally in coursework. As part of the UF community, we also strive to connect our learners with other opportunites for learning programming and data analysis on campus.We typically teach one to three workshops per semester and registration is usuallly announced on the UFII mailing list and the UF graduate student mailing list. Continuing with our efforts to expand computational training to the UF community outside of Gainesville, we now have a program in place to offer workshops at UF Research & Education Centers (with generous support from UF IFAS). The UF Informatics Institute and Biodiversity Institute share a multi-function space on the main floor of the CISE building facing the Marston Science

Library and Century Tower. They generously allow us to use their classroom and meeting facilities as well as provide the staff for catering and logistical support.

143. VALIDATION OF REDUCED INCUBATION TIMES FOR HIGH CONTAINMENT FACILITY BIODECONTAMINATION

Stephan Patrick Willias - University of Florida Division of Environmental Health & Safety; **Mohammed Rashid** - Emerging Pathogens Institute, University of Florida; **Mohammad Siddiqur Rahman Khan** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Jennifer Miller** - University of Florida Animal Care Services; **Gary Heil** - University of Florida Division of Environmental Health & Safety; **Sharon Judge -** University of Florida Division of Environmental Health & Safety; **Karen Gillis** - University of Florida Division of Environmental Health & Safety; **Eric Chesser** - Emerging Pathogens Institute, University of Florida

Sterilants, such as vaporized hydrogen peroxide (VHP), are routinely utilized for high containment facility biodecontamination. Conventional means of sterilant validation rely upon biological indicators comprised of Geobacillus stearothermophilus spores which require a manufacturer-specified 7 day incubation period to determine results. However, the prolonged incubation time is inconsistent with G. stearothermophilus growth kinetics, imposing undue facility downtime that disrupts research progress and imposes a significant financial burden. Within this study, we aim to establish a simple, affordable, and highly reproducible approach to validate a reduced incubation time for VHP facility biodecontamination efforts. In brief, Crosstex biological indicators were enveloped with 0 to 10 layers of cellulose-based tape which impairs VHP penetration and induces hydrogen peroxide decomposition. Duplicate pairs of wrapped biological indicators were staged in parallel along an exposed wall. The experiment was concurrently replicated in two separate rooms per study, with one room staged with a VHP generator and the other subjected to fan-distributed VHP. The entire study was replicated for both “dry” (Steris) and “vaporous” (Bioquell) VHP biodecontamination systems. Following routine VHP facility biodecontamination, one set of indicators was inoculated in accordance with manufacturer guidance for standard growth analysis using colorimetric Crosstex medium; whereas, the

duplicate spore disc was re-suspended in 500 ml of Crosstex growth medium and distributed on TSA plates for recoverable colony forming unit (CFU) enumeration. All biological indicator failures clearly demonstrated growth within 24 hours post-incubation for both VHP systems. As expected, no recoverable CFUs were observed in the biological indicator groups that failed to indicate growth in the standard broth medium. Conversely, recoverable CFUs were detected for all biological indicator groups which displayed growth in the conventional medium. Of the failed biological indicators, the group protected by the lowest layer of tape indicated as little as 100 surviving spores. Taken together, these observations indicate that VHP failures resulting from a low number of viable spores are readily detected well within 48 hours post inoculation. These findings provide robust empirical support for the implementation of biological indicator reduced incubation time determination for facility VHP biodecontamination.

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