Funded by NASA/SurfPlasma Inc., Tamara Revazishvili, Ph.D., works on establishing effectiveness of the Active Plasma Sterilizer (APS) for sterilization in planetary protection. The APS uses Compact Portable Plasma Reactors (CPPRs) to produce surface dielectric barrier discharge (SDBD), a type of cold plasma, which generates and distributes reactive species like ozone used for decontamination. NASA Planetary Protection policies have focused on fungal and bacterial contamination in the International Space Station, where it causes problems in enclosed space. Microbial reduction capability testing has been done by the APS prototype against selected contaminants relevant for planetary protection.
Thus, Aspergillus fumigatus is chosen for establishing sterilization efficacy of APS prototype against fungus.
A. fumigatus is a species of fungus in the genus Aspergillus and is one of the most common Aspergillus species to cause disease in individuals with an immunodeficiency. Sterilization tests were performed with pathogenic A. fumigatus to irradicate, decrease and decontaminate fungal contamination.

EMERGING PATHOGENS INSTITUTE
UNIVERSITY OF FLORIDA

EPI RESEARCH DAY BOOK OF ABSTRACTS 2024

EMERGING PATHOGENS INSTITUTE

RESEARCH DAY

Book of Abstracts | February 2024

Table of contents

Letter from the director

Welcome to the 17th annual EPI Research Day! We are pleased to have a total of 125 abstracts being presented today, with a wide range of topics reflecting the interdisciplinary nature of the research done by EPI members and collaborators. Abstract submissions came from 30 departments in ten UF colleges, as well as from collaborators from other U.S. universities and state and federal agencies. We again have poster competitions for trainees and early investigators (with prizes), information booths from different UF units, and live social media engagement on Instagram and Twitter during the event.
We have the honor of introducing you to two outstanding speakers who will provide keynote talks during our afternoon session:

Albert Icksang Ko, M.D., will be speaking on "Making global health equity work." Dr. Ko is the Raj and Indra Nooyi Professor of Public Health at the Yale School of Public Health and a Collaborating Researcher at the Oswaldo Cruz Foundation, Brazilian Ministry of Health. He served as Chair of the Department of Epidemiology of Microbial Diseases at Yale (2010-2021) after being stationed with the Brazilian Ministry of Health in Salvador, Brazil for 15 years. He and his team have mobilized research and public health responses to multiple epidemics, including meningitis, leptospirosis, dengue, Zika virus infection and associated birth defects, and the COVID-19 pandemic.

Anna P. Durbin, M.D., will be speaking on "Can we get a dengue vaccine over the finish line"? Dr. Durbin is currently a Professor of International Health at the Johns Hopkins Bloomberg School of Public Health, where she has been a member of the faculty since 1999. Her research interests include the human responses to live attenuated vaccines and subunit protein vaccines, with a primary focus on the flavivirus vaccines, particularly those for dengue and Zika virus. Her group has developed two dengue-controlled human infection models (D-CHIM) and two Zika virus CHIMs to down-select candidate vaccines and therapeutics and to better characterize the clinical and immune responses to these viruses; the lead dengue candidate identified, TV003, is nearing completion of a 5-year efficacy trial in Brazil.

We very much appreciate your participation in today's activities – it is going to be a great day!

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

Breakfast and Registration

10:00 AM – 1:00 PM

Poster session
Presenters, please stand by your posters

12:00 PM – 12:45 PM

Lunch

12:45 PM – 1:00 PM

Keynote assembly

1:00 PM – 1:10 PM

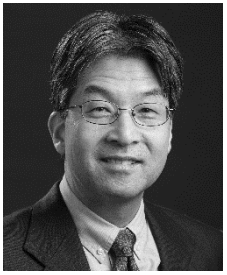
Welcome and Introductions
Dr. J. Glenn Morris, Jr., EPI Director

1:10 PM – 3:15 PM

Keynote speeches

3:15 PM – 4:00 PM

Poster removal



1:10 p.m. – 2:10 p.m.

Albert Icksang Ko, M.D.

Raj and Indra Nooyi Professor of Public Health
Yale University
School of Public Health

"Making global health equity work."

2:10 p.m. – 3:10 p.m.

Anna P. Durbin, M.D.

Professor of International Health
Johns Hopkins University
Bloomberg School of Public Health

"Can we get a dengue vaccine over the finish line?"

01. ASSESSING THE EFFICIENCY OF RT-QPCR IN DISTINGUISHING INFECTIOUS FROM NON-INFECTIOUS NOROVIRUS USING TULANE VIRUS AS A SURROGATE

Razieh Sadat Mirmahdi - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Samantha Lauren Dicker** - Department of Food Science and Human Nutrition, 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

Introduction: Human norovirus is the primary contributor to global viral foodborne illnesses. The lack of a reliable cultivation system requires the utilization of RT-qPCR for subsequent detection and measurement. As a result, scientists use cultivable virus surrogates such as the Tulane virus to extrapolate the environmental fate of human norovirus. Nevertheless, the effectiveness of RT-qPCR in differentiating between infectious and non-infectious particles, as opposed to infectivity assays, remains uncertain.

Purpose: To explore the correlation between the infectivity (plaque) assay and RT-qPCR to quantify norovirus, using Tulane virus as a surrogate.

Methods: Tulane virus lysate was purified through gradient ultracentrifugation and adjusted to a working concentration of 7 log10 plaque-forming units (PFU) per mL in Tris-EDTA buffer (pH = 7.2). The virus stock was serially diluted, and RNA extraction was performed with or without RNase pre-treatment. Subsequently, the extracted RNA was quantified using a probe-based RT-qPCR technique. The conventional RNase pre-treatment was applied to degrade free RNA present in the

virus suspension, thereby enhancing the RT-qPCR detection of intact, presumptively infectious virus particles.

Results: A strong correlation was found between log10 genome copies (GC) and log10 PFU per reaction for both RNase-treated and untreated samples, with a Pearson's product moment correlation coefficient of 0.995. The RNase pre-treated samples showed a 0.2±0.1 lower log10 GC per reaction across the dilutions ($p<0.05$), indicating the integrity of the purified virus stock. Compared to the infectivity assay, RT-qPCR quantified Tulane virus particles at 3.4±0.1 and 3.7±0.1 log10 higher when samples were treated with and without RNase, respectively ($p<0.05$). This emphasizes the sensitivity of RT-qPCR and the possibility of overestimating the number of infectious virus particles. However, the latter assumption does not account for the actual multiplicity of infection when performing a plaque assay.

Significance: The findings of this study will enhance the precision in estimating infectious norovirus particles in food and environmental samples by establishing a correlation between the virus quantification data obtained from probe-based RT-qPCR and the infectivity assay.

02. EMERGING MUTANT VIBRIO CHOLERAE STRAINS FROM THE DEMOCRATIC REPUBLIC OF CONGO REMAIN RESISTANT TO THEIR EVOLVING LYTIC BACTERIOPHAGES

Soung Iballa Francklin Brice - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Juan Guillermo Perez Jimenez** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Meer Taifur Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Felicien Masanga Maisha** - Emerging Pathogens Institute, University of Florida; **Kabangwa Kakongo Senga R** - Appui Medical Integre aux Activites de Laboratoire (AMI-LABO), Goma, Democratic Republic of Congo; **Marco Salemi** - 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; **J. Glenn Morris, Jr.** - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: Lytic bacteriophages (phages) of Vibrio cholerae, the bacterium causing the acute diarrheal disease cholera, cohabitate with Vibrio cholerae in aquatic environments and human intestines. In those niches, phages often infect and kill V. cholerae, thus decreasing their population while selecting phage-resistant mutants. Lytic phages are, therefore, considered a natural modulator of cholera outbreaks and a promising alternative to antibiotics for cholera treatment. It is relevant to study phages' abilities to kill emerging phage-resistant mutants of Vibrio cholerae over time, given the antimicrobial resistance in Vibrio cholerae and the upsurge in cholera outbreaks worldwide, especially in Africa, the most affected region. This study assesses evolution in the lytic phage

genome in the Democratic Republic of Congo (DRC) and the abilities of new phages to prey on previous phage-resistant Vibrio cholerae strains.

Methods: Phages were isolated following centrifugation, filtration of fecal samples from cholera patients, and testing of the resulting fecal filtrates using plaque assay against a known phage-susceptible Vibrio cholerae O1 strain. The susceptibility of 2015-2017 phage-resistant Vibrio cholerae O1 to 2020-2021 phages was assessed via plaque assay.

Whole-genome sequencing was performed with the Illumina MiSeq for 50 cycles for the 2020-2021 phage isolates. Quality control and trimming, genome assembly, and annotation were performed using de novo assembly spades, followed by a rapid genome annotation with Prokka. Pan-genome analysis used Roary software to cluster orthologous genes across multiple viral genomes to identify core genes and unique genetic content among isolates. Single nucleotide polymorphism (SNP) detection was obtained to identify recombinant phages using SNP-sites and Gubbins. Maximum likelihood bootstrapped phylogenetic tree was generated with IQTree with TVMe + ASC +R2 as the best fitting nucleotide substitution model identified by Bayesian information criterion.

Results: Ten phages were isolated and sequenced, 7 in 2020 and 3 in 2021; they were all ICP1 myoviridae phages. Six new phages satisfied the inclusion criteria for the phylogenetic tree, in which they clustered closely with the 2015-2017 DRC phages. Five created a new clade within the DRC phages; the remaining one formed a clade with two previously isolated DRC phages. None of the ten phages could kill the four selected phage-resistant Vibrio cholerae O1 isolates from 2015-2017.

Conclusions: Despite suggestions for evolution in the ICP1 phage genome in DRC between the 2015-2017 and 2020-2021 isolation periods, new phages cannot overcome Vibrio cholerae anti-phage predation mechanisms. Monitoring Vibrio cholerae-phages interaction is critical for understanding cholera transmission in Africa.

03. ESCHERICHIA COLI TRANSFER ONTO AND INTERNALIZATION INTO STRAWBERRIES DROPPED ON PLASTIC MULCH

Claudia A. Pegueros Valencia - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Michelle D. Danyluk** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Loretta M. Friedrich** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: The US FDA Produce Safety Rule prohibits the distribution of dropped-covered produce due to the risk of contamination from the ground and the potential for impact damage to increase the internalization of pathogens. The objective of this study was to evaluate Escherichia coli transfer and internalization potential to strawberries dropped onto plastic mulch from various heights.

Methods: Unwashed strawberries (n=192), randomly selected and weighed, were dropped over both new and used plastic mulch using PVC pipes of varying heights (15.24, 30.48, 60.96, 121.96 cm). Mulch was spot-inoculated with gfp-tagged E. coli (ca. 8 Log CFU) and dried for 1h. Bacterial transfer (BT) to fruit surfaces was assessed via plate count. To measure bacterial internalization (BI), fruit surfaces were sterilized, prior to homogenization and plate counts followed by enrichment to identify the presence of E. coli. Three independent experiments with four replicates were conducted (n=16); BT and BI percentages were calculated. One-way ANOVA found significant differences ($p>0.05$) among scenarios; linear regressions examined correlations between bacterial BT/BI rates and fruit weight.

Results: E. coli survived significantly better during drying on new plastic mulch (7.6 ± 0.25 Log CFU/mulch) than used mulch (6.9 ± 0.58 Log CFU/mulch) ($p<0.05$). CFU transfer to strawberry surfaces was significantly elevated ($p<0.05$) from new mulch (2.62 ± 1.6% to 8.75 ± 3.77%) compared to used mulch (0.018 ± 0.01% to 16.46 ± 15.38). Though internalized bacteria were minimal (less than 0.7 Log CFU/strawberry), the presence of E. coli was detected in strawberries

dropped onto new mulch dropped from 15.24, 30.48 and 121.96 cm, and onto used mulch when dropped from 15.21 and 121.92 cm. No correlation was observed between with BT or BI and weight.

Conclusion: Higher bacterial survival following inoculation and transfer to strawberries was seen from new plastic mulch, emphasizing the importance of not harvesting dropped strawberries.

04. GENOMIC INSIGHTS INTO VIBRIO VULNIFICUS AND ITS POTENTIAL IMPACT ON GLOBAL PUBLIC HEALTH

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Meer Taifur Alam** - Emerging Pathogens Institute, University of Florida; **Katherine Kim** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Sk Nazmus Sakib** - Emerging Pathogens Institute, University of Florida; **Marie-Claire Rowlinson** - Florida Department of Health; **J. Glenn Morris, Jr.** - Department of Infectious Disease, Emerging Pathogens Institute, College of Medicine, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Vibrio vulnificus poses a significant threat to global public health, with its capacity to cause diarrheal, wound, and lethal systemic infections with mortality rates over 50%. Despite decades of research, defined virulence factors and the mechanism of pathogenesis of V. vulnificus remain elusive. V. vulnificus has three biotypes (I, II, and III) based on phenotypic traits. V. vulnificus infects a wide range of hosts, including humans, fish (Oysters, Tilapia), and marine and estuarine aquatic reservoirs. The pathogen thrives in elevated temperatures and salty water. Recent climate change has correlated markedly with the increase of V. vulnific infections to the geographic and seasonal expansion globally, underscoring the urgent need for comprehensive research and preventive strategies for V. vulnificus diseases. This study aimed to understand the

pathogenesis and genomic attributes of V. vulnificus strains isolated from various hosts. We sequenced 27 isolates from Tilapia fish and humans then compared to 60 genomes reported by the Florida Department of Health over 2022 and 2023. Through phylogenetic analysis, we found that strains with different biotypes often share similar genomic clusters, indicating a complex interrelation of genetic factors across biotypes. Remarkably, all strains carried genes encoding tetracycline resistance, while a strain (AA101) carried resistance genes for streptomycin (aadA13), chloramphenicol (catA2), and sulfonamides (sul1). All isolates carried critical virulence genes encoding the repeats-toxin (rtxA, rtxB, and rtxC), cytolytic hemolysin (vvhA), and adhesins (ilpA, ompU). Furthermore, we observed widespread horizontal gene transfer events, mediated by transposase, integrons, and phage-related genes, indicating a dynamic genomic landscape that facilitates adaptation and resistance. This study reveals the genomic traits of V. vulnificus that facilitate its adaptation to environments, hosts, and infectious capabilities, underscoring the critical need for comprehensive global health strategies to address the expanding threat posed by V. vulnificus.

05. IDENTIFICATION OF BIFIDOBACTERIA WITH PROBIOTIC POTENTIAL FOR THE TREATMENT OF SEPTICEMIA IN DAIRY CALVES

Jonathan Crabill - Department of Biology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Yuting Zhai** - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Bugil Choi** - Emerging Pathogens Institute, University of Florida; **Peixin Fan** - Mississippi State University; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Septicemia, diarrhea, and pneumonia are the primary causes of mortality in dairy calves. Septicemia often arises from diarrhea, which can be caused by bacterial infections such as E. coli and Salmonella, typically treated with antibiotics. Maintaining a healthy gut microbiome is essential to prevent the colonization of these pathogens. To develop probiotics that can aid in rebuilding the microbiome in diarrheic calves, we aimed to isolate microorganisms from healthy calves. A total of 130 fecal samples from dairy calves aged 1 to 28 days were collected from the rectal anal junction, including 47 samples from healthy calves and 83 from calves with diarrhea/septicemia. Through 16S rRNA gene sequencing analysis, we found a strong negative correlation between the abundance of Bifidobacterium and septicemia incidence in calves, suggesting that specific Bifidobacterium strains could be effective probiotics to prevent sepsis in diarrhea calves. From healthy calves, we isolated 54 B. longum and 6 B. pseudocatenulatum strains. By comparing our B. longum genomes to 50 other publicly available B. longum genomes, we found host- and environment-related genomic features. Using in silico analysis, we tested whether these strains carry antimicrobial resistance genes (ARGs). All tested strains carried ARGs, suggesting these strains acquired them from antibiotic use on dairy farms. In vivo antibiotic susceptibility tests will be conducted to select probiotic candidates before we conduct animal trials. This research not only promises to enhance the health and welfare of dairy calves but also

stands as a potential change in farm management strategies, paving the way toward more sustainable dairy farming practices.

06. IMPACT OF SUGAR-REGULATED O-ANTIGEN MUTATIONS ON SALMONELLA RESISTANCE

Hailey Mangio - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Shifeng Wang** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Ashley Pitzl** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Roy Curtiss III** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

The O-antigen is a polysaccharidic component of lipopolysaccharide (LPS), located on the outer membrane of Gram-negative bacteria. It is a critical surface structure that influences bacterial pathogenesis and contributes to resistance to serum, particularly to heat-sensitive complement components. The O-antigen ligase enzyme, waaL, is involved in the biosynthesis and attachment of O-antigen to the core of LPS, and the pmi gene is required to synthesize the O-antigen side chain. In this project, Salmonella strains with waaL gene expression regulated by the sugars arabinose and rhamnose and with pmi mutations requiring mannose for O-antigen side chain synthesis were generated. Single-sugar and double-sugar regulated strains were compared by analyzing motility, serum resistance, and acid tolerance. Motility was assessed by measuring swimming zones on motility test plates. Serum resistance was measured by conducting colony-forming unit counts after 2-hour incubations of bacteria in normal human serum (NHS) and heat-inactivated human serum (IHS). Additionally, 48-hour growth curves based on strains' optical density in 10% NHS and IHS were evaluated. Acid tolerance was also tested by measuring 48-hour bacterial growth curves in E-mediums with varying pH. Mutation-mediated variations in O-antigen synthesis were found to influence differences in motility, serum resistance, and acid tolerance among the strains tested. This study contributes to current understandings of the impact of surface modifications on Salmonella

pathogenesis and informs directions for recombinant attenuated Salmonella vaccine development. Subsequent research could test additional measures such as bile resistance and examine effects of the mutations on Salmonella virulence in animal models.

07. IMPLICATIONS OF SYMBIOSIS ON LISTERIA MONOCYTOGENES BIOFILM ON FOOD CONTACT SURFACES

Tingting Gu - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, University of Florida; **Boce Zhang** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, University of Florida

We investigated Listeria monocytogenes biofilm formation in cocktail cultures under various conditions. We found L. monocytogenes biofilm formation is greatly affected by symbiotic microorganisms, and their symbiosis can be influenced by the environment.

Introduction: L. monocytogenes can adhere to food contact surfaces and form highly persistent biofilms. L. monocytogenes biofilm can be significantly affected when it forms cocktail biofilms with other microorganisms, especially good biofilm formers. To investigate how this symbiosis affects L. monocytogenes' persistence, the study developed cocktail biofilms of L. monocytogenes with other common isolates in produce processing facilities.

Methods: Stainless steel 304 coupons (SS304) as standard food contact surfaces were used with four types of noncoated surface topographies: 1) coupons with commercial native/bare finish (SS304-B), 2) coupons with commercial #4 brushed finish (SS304-4), 3) coupons modified with micropillars (SS304-Dot), and 4) coupons modified with microlines (SS304-Line). The study included coupons with and without Dursan coating, a previously identified fouling-resistant agent against monospecies L. monocytogenes biofilm. The study evaluated the symbiosis in dual-species cocktail biofilm (L. monocytogenes + Escherichia coli O157:H7; L. monocytogenes + Pseudomonas fluorescence; L. monocytogenes + Ralstonia insidiosa) and four-species cocktail biofilm (L. monocytogenes + E. coli O157:H7 + P. fluorescence + R. insidiosa). L.

monocytogenes biofilms were cultivated in monospecies and cocktail species on different SS304 coupons for 7 days at 4 °C in lettuce juice extract to simulate the produce processing conditions.

Results: Among dual-species cocktail biofilms, P. fluorescence had a strong synergistic effect on L. monocytogenes biofilm formation on all SS304 coupons. E. coli O157:H7 and R. insidiosa showed synergistic or antagonistic symbiosis with L. monocytogenes on different coupons, suggesting substrate properties can influence symbiotic relationships in cocktail biofilm. In the four-species cocktail biofilm, synergistic symbiosis with L. monocytogenes was consistently observed under all conditions. The results suggest that symbiosis in biofilm can be impacted by cocktail species, organic loads, surface chemistry, and surface topography.

Significance: Symbiosis is critical in L. monocytogenes biofilm formation and can have significant food implications. Synergistic symbiosis with L. monocytogenes should be mitigated to avoid negative food safety implications. Antagonistic symbiosis may indicate potential intervention strategies against L. monocytogenes biofilm.

08. INTERVIEWING SURVIVORS AND TEACHING STUDENTS: REMEMBERING SOCIETAL RESPONSES TO INFECTIOUS DISEASE OUTBREAKS

Nina Stoyan-Rosenzweig - Center for African Studies, College of Medicine, University of Florida; **Dylan Lucaksa** - College of Liberal Arts and Sciences, University of Florida

This poster discusses a project conducting oral history interviews with survivors of childhood "poliomyelitis", making these and other polio "narratives" available, and teaching undergraduate courses on the history of social and individual experiences and responses to "infectious disease outbreaks". It describes the interviews, publications and digital collection, and the classes team taught with undergraduates that explore the cyclical response to infectious disease epidemics throughout history.

09. MOLECULAR EVOLUTIONARY ANALYSIS OF VIBRIO CHOLERAE O1 IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC): ISOLATION OF STRAINS FROM THE AQUATIC ENVIRONMENT AND CHANGING SEROGROUP PATTERNS

Juan Guillermo Perez Jimenez - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Background: From 2017-2021 the DRC reported 149,154 cholera cases and 3,239 deaths; in 2021, DRC had the third highest reported number of cholera cases in the world. A major hotspot for cholera within this region has been the city of Goma located in North Kivu Province along the northern shore of Lake Kivu. We have monitored V. cholerae epidemiology and evolutionary genomics in the Goma region since 2015, working in collaboration with provincial health authorities and AMI-LABO, the provincial public health laboratory.

Methods: Whole genome sequencing, mapping and high-quality single-nucleotide polymorphism calling was performed for 165 V. cholerae O1 isolates from 2019 to 2022; 24 environmental isolates, and the rest from clinical cases seen at cholera treatment centers. This analysis was

performed including 24 clinical isolates from 2015-2017 previously reported by our group and 77 complete genomes from SRA. Maximum likelihood was conducted using IQ-Tree and phylogeography tree reconstruction were performed with BEAST version 1.10.4.

Results: 24 isolates were from community water sources used by the household of an index cholera case, and from Lake Vert, a natural lake located on the western outskirts of Goma. All environmental isolates were serotype Ogawa and were susceptible to our previously reported DRC ICP1 bacteriophage. Isolates from 2015-2017 were primarily within a major lineage previously designated as Goma lineage II and were serogroup Inaba (albeit with emergence of a few Ogawa strains in 2020). However, by 2020 virtually all clinical and environmental isolates clustered within a different major lineage, designated as Goma lineage I/sequence type 69 which dates to the first reported outbreaks of cholera in DRC during 2008-2009. These isolates were predominantly Ogawa, with all environmental strains clustering within a single sublineage that included the only Hikojima strains identified. Molecular clock analysis suggested that the two major lineages diverged in 1999 with the highest 95% posterior density interval of February 1993 to April 2001. Phylogeographic reconstruction based on location of collection - Nord Kivu and South Kivu - revealed intermixed circulation of lineages in the Goma region.

Conclusion: Cholera is present in the environment in the Goma region, including sites linked with known cholera cases; interestingly, all environmental strains were serogroup Ogawa, and were within a single sublineage. We saw a shift in major lineage and serogroup among clinical isolates, from Goma lineage II/Inaba back to Goma lineage I/sequence type 69 Ogawa strains. Evolutionary drivers for this shift remain to be determined.

10. PREDICTING SALMONELLA SHEDDING AT SLAUGHTER: A FEASIBLE STEP TOWARDS THE REDUCTION OF INFECTION ATTRIBUTED TO PIGS

Raul Carlos Mainar Jaime - Emerging Pathogens Institute, University of Florida; **Maria Bernad-Roche** - University of Zaragoza, Spain; **Clara M Marin-Alcala** - Centro de Investigacion y Tecnologia Agroalimentaria de Aragon, CITA, Zaragoza, Spain; **Ignacio de Blas** - University of Zaragoza, Spain; **Alberto Cebollada-Solanas** - University of Zaragoza, Spain

Introduction: salmonellosis remains a significant global cause of foodborne outbreaks, with pigs being a primary source of human infection. Contamination of pork by Salmonella is a major concern for abattoirs, linked to the presence of the bacteria in pig feces during slaughter. Predicting the risk of Salmonella shedding in pigs arriving at the slaughterhouse could assist in reducing abattoir and carcass contamination.

Methods: thirty pig fattening units (1000-2000 pigs/unit) were selected. A biosecurity questionnaire (https://biocheck.ugent.be/en/questionnaires/pigs) was administered on each farm. One month prior to slaughter, 50 pigs per unit were selected, tagged, and bled to detect Salmonella antibodies (Herdcheck Swine Salmonella, IDEXX Laboratories, Westbrook, ME, US). Pooled floor fecal (PFF) samples from 10 pens per unit were also collected for Salmonella detection (ISO 6579:2002/Amd 1:2007). At slaughter, intestinal content (IC) was collected from the colon of each pig for Salmonella shedding detection. Salmonella isolates from farms and IC were compared using PFGE. A predictive model for Salmonella shedding at slaughter was developed with 2/3 of the selected pigs using random-effects logistic regression. The model included Salmonella shedding as the dependent variable, with serology, farm biosecurity score, % of Salmonella positive pens, season, and distance to slaughter as independent variables. Farm was treated as a random (grouping) variable. The remaining pigs were used for model validation.

Results: 1,341 IC samples were collected at the slaughterhouse (44.7 pigs/farm). The mean seroprevalence among the 30 units was 31.4%

(range 2.3%-87%), indicating widespread Salmonella circulation, as only six farms had seroprevalences below 10%. Salmonella was found in PFF samples in 43.3% of the units, with 53.8% of them having over 30% of pens positive for Salmonella. Overall, 316 (23.6%) pigs shed Salmonella at slaughter, mainly S. Typhimurium and its monophasic variant. PFGE analysis revealed genetic relatedness between isolates from IC and PFF from the corresponding unit. Random-effects logistic regression analysis identified three significant variables associated with Salmonella shedding: serology (OR=1.75), percentage of Salmonella-positive pens (OR=5.46), and internal biosecurity score (OR=0.25). The predictive and validation models showed similar performance. A simplified model with the latter two variables slightly improved overall prediction.

Conclusions: a model incorporating a pigs' serological results, farm's internal biosecurity level, and the percentage of Salmonella-positive pens can predict Salmonella shedding at the abattoir. However, a simpler model, comprising only the internal biosecurity level and the percentage of positive pens, may yield comparable results with significantly less data collection effort.

11. QUANTIFICATION OF SHIGELLA USING QPCR TARGETING IPAH IN CHILDREN UNDER 5 YEARS FROM A CASE-CONTROL STUDY IN HAITI

Ishae Sriguha - Department of Pediatrics, Emerging Pathogens Institute, University of Florida; **Saradiya R Kuyt Scott** - Department of Pediatrics, Emerging Pathogens Institute, University of Florida; **Molly B Klarman** - Department of Pediatrics, University of Florida; **Youseline Cajusma** - Department of Pediatrics, University of Florida; **Md Abu Sayeed** - Department of Environmental and Global Health, University of Florida; **Emilee Cato** - Department of Pediatrics, University of Florida; **Valery Madsen beau de Rochars** - Department of Health Services Research, Management and Policy, University of Florida; **Chantale Baril** - State University of Haiti; **Eric Nelson** - Department of Pediatrics, University of Florida

Background: Diarrheal disease is a major cause of mortality among children <5 years. In Haiti, the under-five mortality rate is 59 deaths per 1000 live births. Globally, the GEMS and MAL-ED studies have shown that Shigella spp. are a leading cause of symptomatic disease among patients in the community and admitted to hospitals. However, there are no data from case-control studies to support this finding in Haiti.

Methods: We conducted a case-control study within a larger cross-sectional study investigating healthcare-seeking behavior in Haiti. Our objective was to use qPCR to detect and quantify Shigella spp. from children <5 years that were symptomatic versus asymptomatic for diarrhea at the household level. Participant recruitment was completed using a method randomized by geospatial location in the semi-rural regions of Gressier and Leogane Haiti. Stool samples were obtained via rectal swab and frozen in normal saline at -80C; qPCR was conducted for Shigella spp. (ipaH). The positive control of 16S for eubacteria was included. For statistical analysis, two definitions for cases were considered: (i) Cases for 'diarrheal symptoms' were defined as reporting diarrheal symptoms ≤7 days ago. (ii) Cases for 'acute diarrhea' were defined as reporting diarrheal symptoms ≤7 days ago and ≥3 loose stools in the past 24 hours and onset <7 days ago. An odds ratio was used to

compare rates of disease between cases and controls using R (version 4.3.2).

Results: Of the 868 households screened, 568 were enrolled with 794 children. Of these children, 732 met inclusion criteria for the case-control analysis The rates of Shigella detection among cases with 'diarrhea symptoms' and controls were 11.4% (22/193) and 6.1% (33/539), respectively. The rates of Shigella detection among cases with 'acute diarrhea' and controls were 18.6% (8/43) and 6.8% (47/689), respectively. The odds of testing positive for Shigella increased by 97% among cases with 'diarrheal symptoms' (OR=1.97; 95% CI 1.12 to 3.48) and increased by 212% among cases with acute diarrhea (OR=3.12; 95% CI 1.37 to 7.11). Minor differences were detected with marginal statistical significance. Measures of potential confounding factors (e.g., age, malnutrition, wealth) were not appreciably different.

Conclusion: Within this case-control study in Haiti, Shigella spp. detection was 7.5% overall. There was a meaningful increased odds of symptomatic disease when Shigella spp. were detected. These results are consistent with prior global studies. There is a need to prioritize surveillance of shigellosis and treatment as a public health priority in Haiti.

12. SALMONELLA'S TRANSFER POTENTIAL BETWEEN INTACT AND DAMAGED TOMATOES AND NEW AND USED HARVEST BIN MATERIALS DURING HARVESTING

Mari Schroeder - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Michelle D. Danyluk** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: Tomatoes have been associated with Salmonella outbreaks in the U.S. The purpose of this study was to evaluate Salmonella's transfer potential between intact and damaged tomatoes and new and used harvest bin materials.

Method: Intact and damaged tomatoes, and new and used HDPE, wood, or cardboard coupons were spot inoculated with a Rifampicin resistant Salmonella cocktail (10^5) and dried for 1h. Uninoculated and inoculated items were placed into contact, compressed with a 1lb weight, for 10min, 3, 6, and 24h. Salmonella populations on both items were enumerated following a shake, rub, shake (30s each), dilutions, plating onto non-selective media with Rifampicin, and incubation (35°C, 24h). The experiment was replicated 3 times with 3 samples (n=9). The CFU/mL transferred to the uninoculated surface was divided by the CFU/mL on the inoculated surface and reported as log% TCs.

Results: Salmonella transfer to (range 0.897-2.005 log%) and from (0.250-2.044 log%) damaged tomatoes and used bin materials were significantly ($P \leq 0.05$) greater than the transfers to (-1.111-0.000 log%) and from (-0.847-0.000 log%) intact tomatoes and used bin materials, across all time points. There were no significant differences in transfer between intact and damage tomatoes, to (1.946-2.092 log%) and from (823-2.096 log%) new HDPE, across all contact times. There were significant ($P \leq 0.05$) transfer differences between intact and damaged tomatoes to new: wood at 3 (1.387 and 2.158 log%), 6 (0.000 and 2.107 log%), and 24h (0.000 and 2.069 log%), and to cardboard at 6h (0.000 and 2.259 log%). There were significant ($P \leq 0.05$) transfer differences between intact and damaged tomatoes from new wood at 6h (0.000 and 1.984 log%), and cardboard at

10min (1.930 and 0.000 log%), 3 (1.216 and 0.000 log%), and 6h (0.000 and 2.383 log%).

Conclusion: Transfer potential increases with damaged tomatoes and used surfaces; damaged tomatoes should not be harvested or picked up, and harvest bins should be maintained.

13. SEROTYPE-DRIVEN ENVIRONMENTAL PERSISTENCE OF VIBRIO CHOLERAE O1 IN HAITI

Sk Nazmus Sakib - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Meer Taifur Alam** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **J. Glenn Morris, Jr.** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Afsar Ali** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: Cholera, a profuse secretory diarrheal disease, remains a major public health threat in countries lacking safe drinking water, improved sanitation, and hygiene. Toxigenic Vibrio cholerae O1, ubiquitous in aquatic reservoirs, is responsible for cholera. We are monitoring cholera epidemiology in Haiti since its introduction in 2010; During our surveillance, we isolated a total of 707 and 63 confirmed V. cholerae O1 strains from clinical and environmental sources, respectively. We identified 31 (49.2%) non-toxigenic O1 strains that correlated with emergence of Inaba serotype in 2015 in Haiti. Specifically, we observed that Inaba strains isolated from aquatic reservoirs tended preferentially to be non-toxigenic and PS serotypes suggesting that Haiti aquatic environment may not be supportive of toxigenic Inaba strains promoting declining of cholera cases and eventual cholera quiescence. To thrive in hostile aquatic environments, V. cholerae must adopt many survival strategies, including biofilm formation, phage susceptibility and survivability in nutrient-poor condition. We hypothesized that various

serotypes of toxigenic and non-toxigenic V. cholerae O1, regardless of their origin, differentially produce biofilm, exhibit lytic phage susceptibility and survivability in nutrient-poor condition.

Methods: We have assessed the comparative biofilm formation and lytic phage susceptibility test among a large number of clinical and environmental strain from Haiti over several years. We also performed genetic manipulation resulting in the conversion of Ogawa to Inaba and Inaba to Ogawa serotype; these converted strains were compared to their respective wild-type strains concerning biofilm formation and rugose conversion in rugose-inducing condition. We also performed microcosm (mimicking aquatic environment) studies using estuarine water collected from Haiti.

Results: Data presented in this study show that Inaba strains are poor biofilm formers compared to Ogawa and PS serotype strains. Data from this study also show that Inaba converted to Ogawa produced biofilm comparable to wild-type Ogawa donor strain. Similarly, Ogawa converted to Inaba produced no biofilm and was comparable to wild-type Inaba donor strain. Strikingly, all non-toxigenic O1 strains (Ogawa, Inaba, and polyvalent-positive) were resistant to lytic phage infection. Results of microcosm studies indicated decreased Inaba serotype persistence compared to Ogawa serotype underscoring the weakness of Inaba serotype in environmental persistence in Haitian environmental reservoirs.

Conclusion: Our data support our hypotheses that Inaba serotype strains are weak biofilm former and thus poor or non-adaptable to Haitian aquatic environments. Our study clearly indicates that environmental factors either facilitate or inhibit V. cholerae's serotype-based persistence and eventual endemic cholera transmission particularly in Haiti.

14. UNDERSTANDING GENETIC FEATURES ASSOCIATED WITH HOST SPECIFICITY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BY A MACHINE LEARNING APPROACH

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Ting Liu** - Emerging Pathogens Institute, University of Florida; **Simone Marini** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Mattia Prosperi** - Department of Epidemiology, College of Medicine, University of Florida; **Christina Boucher** - College of Engineering, University of Florida; **Kartikeya Cherabuddi** - Department of Infectious Disease, College of Medicine, University of Florida; **Nicole Iovine** - Department of Infectious Disease, College of Medicine, University of Florida; **J. Glenn Morris, Jr.** - Department of Infectious Disease, 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

Methicillin-resistant Staphylococcus aureus (MRSA) poses a significant threat to public health and veterinary medicine due to its adaptability across diverse environments and hosts. Nonetheless, the underlying genetic characteristics enabling MRSA to thrive in various settings remain poorly understood. In this study, we aimed to unravel the host-associated genetic features using a machine learning (ML) approach. Our study encompassed the analysis of 2,559 MRSA genomes from human and non-human hosts. By utilizing a source-attribution ML model based on the Support Vector Machine algorithm, we successfully predicted the origin of MRSA strains based on their genomic contents, which emphasized the pivotal role of genetic features in determining MRSA host specificity. The identified crucial genetic features included recombinases, virulence factors, and immune modulators, most of which were associated with prophage. Specifically, phiN315-like prophages demonstrated a high

prevalence among MRSA strains sourced from humans. Our in vitro experiments revealed that excision of phiN315-like prophages led to enhanced MRSA pathogenesis in hosts, mediated by enhanced expression of virulence factors. In summary, our ML analysis revealed specific genetic features associated with the host specificity of MRSA. While MRSA has exhibited as a host generalist throughout its evolutionary history, our findings underscore that MRSA possess distinct genetic features that facilitate host specialization.

15. A RECOMBINANT PROBIOTIC PROTECTS MICE AGAINST INFLUENZA A INFECTION-INDUCED IMMUNOPATHOLOGY

Govindaraj Anumanthan - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Ananta Prasad Arukha** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Ayalew Mergia** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Bikash Sahay** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida

Seasonal Influenza causes millions of hospitalizations and thousands of deaths in the USA. The conventional preventive measure for influenza A is seasonal vaccination, which has varied protection in different groups of individuals from 40-75%. Antivirals are used in cases diagnosed early. Otherwise, primarily symptomatic treatment has been employed. Here, we propose a recombinant probiotic that prevents Influenza disease by boosting the host's immunity. The Surface layer protein A(SlpA) of Lactobacillus acidophilus expressing Lactococcus lactis (R110) has been a proven immunomodulator in the gut under human clinical trials. We found that oral feeding of R110 recruits regulatory T cells and prevents neutrophils, a key mediator for lung inflammation. Additionally, we report heightened antigen-specific CD8 T cells at the later stage of the disease. Further studies are in progress to develop a mechanistic understanding of these events.

16. CONCENTRATING VIABLE AIRBORNE PATHOGENS USING A VIRTUAL IMPACTOR WITH A COMPACT WATER-CONDENSATION AIR SAMPLER

William Vass - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Sripriya Nannu Shankar** - Department of Environmental and Public Health Sciences, University of Cincinnati; **John Lednicky** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Morteza Alipanah** - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida; **Braden Stump** - Aerosol Devices, Inc.; **Patricia Keady** - Aerosol Devices, Inc.; **Z. Hugh Fan** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Chang-Yu Wu** - Department of Chemical, Environmental, and Materials Engineering, University of Miami

Introduction: Pathogens can be collected from air and detected in samples by many methods. However, merely detecting the presence of pathogens does not answer whether they can spread disease. To fully assess health risks from exposure to airborne pathogens, the infectivity of those agents must be assessed. Air samplers which operate by growing particles through water vapor condensation and subsequently collecting them into liquid have proven effective at conserving the viability of microorganisms.

Method: We present a study that assessed performance improvement of one such sampler, BioSpot-GEM™ (GEM), gained by augmenting it with an upstream virtual impactor (VI) designed to concentrate particles in aerosols. We collected Escherichia coli (E. coli) and human coronavirus OC43 (OC43) using GEM and compared amounts of bacteria and virus collected with the VI to amounts without the VI. Metrics assessed were live bacteria, viable virus, and virus quantifiable by RT-qPCR.

Results: We demonstrate that a GEM integrated with a VI improved the collection of live E. coli by a median Concentration Factor (CF) of 1.59 and increased the recovery of viable OC43 by a median CF of 12.7 as compared to the sampler without the VI. Our results also show that OC43

can be concentrated in this way without significant loss of infectivity. We further present that the small GEM can collect live E. coli at an efficiency comparable to the larger BioSpot-VIVAS™ bioaerosol sampler.

Conclusions: Our analyses show potential benefits toward improving the collection of viable pathogens from the air using a more portable water condensation-based air sampler while also highlighting challenges associated with using a VI with concentrated bioaerosols. This work can aid further investigation of VI usage to improve the collection of pathogens from air ultimately to better characterize health risks associated with airborne pathogen exposures.

17. FECAL SHEDDING OF SARS-COV2 IN INFANTS BORN TO SARS-COV2 POSITIVE MOTHERS: A PILOT STUDY

Dylan K. P. Blaufus - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Karen M Kalanetra** - Department of Food Science and Technology, University of California, Davis; **Rosa Pesavento** - Department of Pediatrics, University of California Davis, Sacramento, CA, USA; **Pranav Garlapati** - Department of Pediatrics, University of California Davis, Sacramento, CA, USA; **Brittany Baikie** - Department of Food Science and Technology, University of California, Davis; **Kara M Kuhn-Riordan** - Department of Pediatrics, University of California Davis, Sacramento, CA, USA; **Mark A. Underwood** - Department of Pediatrics, University of California Davis, Sacramento, CA, USA; **Diana H. Taft** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: Fecal shedding of SARS-CoV-2 correlates with gastrointestinal symptoms. The gut microbiota are associated with resistance to enteric pathogens; COVID19 is associated with alterations to the gut microbiome. We hypothesized that the gut microbiome of infants born to SARS-CoV-2+ mothers differs between infants with and without fecal shedding of the virus.

Methods: We enrolled 10 infants born to SARS-CoV-2+ mothers. We used qPCR on fecal RNA to test SARS-CoV-2 and 16S rRNA gene sequencing to the V4 region to assess the gut microbiome. Infant SARS-CoV2 status from

nasal swabs was abstracted from the medical record. Sequencing reads were analyzed using QIIME2. Statistical analyses were completed in R.

Results: Of the 10 included infants, 9 were tested for SARS-CoV-2 by nasal swab with 1 testing positive. Four infants, including the nasal swab positive infant, had at least one sample with detectable levels of SARS-CoV2 fecal shedding. Detection of both SARS-CoV-2 genes in feces was associated with increased gut alpha diversity compared to no detection by a linear mixed effects model ($p<0.001$). Detection of both SARS-CoV2 genes was associated with increased levels Erysipelotrichaceae, Lactobacillaceae, and Ruminococceae by MaAsLin2.

Conclusion: Fecal shedding of SARS-CoV-2 occurs in infants who test negative on nasal swabs and is associated with differences in the gut microbiome.

18. GENOMIC EPIDEMIOLOGY OF RHINOVIRUS BEFORE AND DURING THE COVID-19 PANDEMIC

Julia Paoli - 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; **Nidia Trovao** - NIH Fogarty International Center; **Sana Tamim** - NIH Pakistan

Introduction: Human rhinovirus (HRV) is the causative agent of > 50% of cold-like illnesses worldwide and responsible for billions of dollars in economic impact each year due to lost productivity. During the 2020-2023 COVID-19 pandemic, strict isolation measures in the initial six months suppressed HRV infections worldwide. Unlike other non-SARS-CoV-2 respiratory viruses, HRV cases returned to pre-pandemic levels in mid-2020, likely due to the reopening of schools in fall 2020 in tandem with the loosening of social restrictions. However, little is known about the impact of these restrictions on the evolutionary history and spatio-temporal dynamics of the virus.

Method: We analyzed the recombination patterns of all publicly available geographical- and time-stamped HRVs -A, -B, and -C full genomes, VP1, and VP4 genes with RDP5. We reconstructed the viral evolutionary history using a maximum likelihood approach implemented in IQ-Tree to shed light on HRVs transmission dynamics.

Results: We obtained 9,225 HRV-A sequences, 1,633 HRV-B sequences, and 6,983 HRV-C sequences from 65 countries worldwide between 1980-2023. Oceania and South America were under-sampled. Kenya (19%), USA (15%), and Germany (12%) submitted the majority of sequences. 84% of sequences were VP4, and only 10% were full genomes. Pre-COVID-19, 15,981 sequences were submitted. During the COVID-19 pandemic, 1,860 sequences were submitted: 56% of the sequences were collected in USA, particularly in Washington and Arizona. Evidence of recombinant sequences was found: 64 for HRV-A full genome, 25 for HRV-B full genome, and 2 for HRV-C VP1. Genomic surveillance was found to peak in times of ongoing respiratory viral outbreaks. Phylogenetic analysis shows geographic intermixing.

Conclusions: Our findings highlight a need for greater genomic surveillance worldwide, particularly in Oceania and South America. We found that surveillance was linked to specific research efforts in select regions which may bias our understanding of global HRV circulation and evolution. Moreover, the lack of sequencing between 2022-2023 poses a challenge for assessing post-pandemic viral dynamics.

19. LOW PREVALENCE OF SARS-COV-2 IN BOTH FARMED AND FREE-RANGE FLORIDA WHITE-TAILED DEER

Savannah Grace - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Kristen Wilson** - Department of Wildlife Ecology and Conservation, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, University of Florida; **Ruiyu Pu** - Department of Veterinary Medical Sciences, College of Veterinary Medicine, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **John Lednicky** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida; **Juergen Richt** - Kansas State University; **Maureen Long** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida

Introduction: SARS-CoV-2, the novel coronavirus that causes COVID-19 in humans, exhibits the ability to infect a diverse range of animal species, including both domestic and wild animals. Among these species, white-tailed deer (WTD) are particularly concerning due to their genetic similarity to humans in the ACE2/S protein binding motif, coupled with their status as the most abundant and widely distributed large mammal in North America. Previous research has documented the presence of SARS-CoV-2 in both farmed and free-range WTD across the United States and Canada, suggesting possible transmission between humans and WTD. Given their frequent close interactions with humans, farmed white-tailed deer (WTD) are expected to have a higher risk of exposure to SARS-CoV-2, potentially leading to a greater rate of infection compared to their free-ranging counterparts. Here, we aimed to address these assumptions by assessing the prevalence of previous or current SARS-CoV-2 infection in both farmed and free-range WTD in Florida during the COVID-19 pandemic.

Methods: Respiratory secretion swabs (N=401), lung tissue (N=260), retropharyngeal lymph nodes (N=666), and serum (N=433) were collected from both farmed and free-range WTD in Florida between November 2019 and December 2022. Samples were analyzed to detect current SARS-CoV-2 infection by evaluating the presence of viral RNA using RT-qPCR, or to determine previous infection through the detection of SARS-CoV-2 neutralizing antibodies using multiple virus neutralization assays.

Results: Our findings indicate a low prevalence of SARS-CoV-2 infection in both farmed and free-range WTD in Florida. Among all farmed deer sampled, less than 1% were currently infected at the time of sample collection. Specifically, the positive detection rate was 0.67%, constituting 3 positives out of 443 individual deer when adjusting for paired samples (95% CI: 0.13—2.11%). Similarly, the prevalence of infection in free-range WTD was only 0.76% (4/526, 95% CI: 0.76—2.01%). Serology results showed that among the farmed deer sampled, none possessed virus-neutralizing antibodies (0/352), while only one free-range WTD did (3.45%; 95% CI: -0.84—18.63%).

Conclusions: Our findings suggest that the risk of SARS-CoV-2 spillover from infected humans to WTD in Florida may be minimal. Both farmed and free-range WTD showed limited evidence of exposure, raising questions about potential regional differences that could contribute to variations in the prevalence of SARS-CoV-2 among WTD populations across North America.

20. ON THE SPOT COLLECTION AND DETECTION OF AIRBORNE HCOV-OC43 VIRUS

Matt Jansen - Department of Biomedical Engineering, Emerging Pathogens Institute, College of Engineering, University of Florida

Rapid and accurate point-of-care detection (POC) is necessary to reduce the transmission of contagious diseases especially as infection numbers increase. We have developed a valve-enabled, paper-based sample preparation device integrated with isothermal amplification and adapted for collection and detection of these circulating viruses. By utilizing water-based condensation, we are able to increase the size of the aerosol particles and collect them for analysis. The device incorporates (1) virus lysis and RNA enrichment, enabled by ball-based valves for sequential delivery of reagents with no pipet requirement, (2) reverse transcription loop-mediated isothermal amplification, carried out in a battery-powered coffee mug, and (3) colorimetric detection. The device has been able to detect on the spot virus concentrations as low as 40 GEs per microliter in HCoV-OC43. Our results show the potential of the device for POC detection of circulating aerosol viruses.

21. RHINOVIRUS-C INFECTION AS A CAUSE OF A FALSE-POSITIVE RESULT IN A SARS-COV-2 ANTIGEN TEST

Tracey L Moquin - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Disease, 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

The gold standard for COVID-19 testing has been detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA through reverse transcriptase polymerase chain reaction (RT-PCR). But with the perception that the COVID-19 pandemic is now over, there are few laboratories that perform RT-PCR tests. Instead, rapid at-home lateral flow antigen tests are now the primary diagnostic method for COVID-19 due to their widespread availability and ease of use. The subject of this case was a 20-year-old male who previously had COVID-19. Two months following full recovery, he again began exhibiting clinical signs of acute respiratory illness including sore throat, cough, and nasal congestion. He tested positive for COVID-19 at a local clinic that used a BinaxNOW™ COVID-19 Antigen Self-Test. We obtained a nasal swab specimen and performed RT-PCR. The sample was negative for SARS-CoV-2 RNA using primers aimed at the N and pol genes. The sample was then analyzed using a BioFire multiplex PCR Film Array Respiratory 2.1 panel, which detected Rhinovirus/Enterovirus. Attempts to culture the virus were unsuccessful in four cells lines. Next, RNA was extracted from the nasal swab specimen and sequenced using an Illumina NextSeq platform, which recovered a nearly complete Rhinovirus-C genome. Our findings highlight the need for confirmatory testing via RT-PCR or virus culture for diagnosing COVID-19. False-positive results via antigen rapid tests skew treatment methods and potentially undermine public perception of the efficacy of the COVID-19 vaccine.

22. SARS-COV-2 SURVEILLANCE AMONG US NATIVE WILD MAMMALS ENTERING REHABILITATION FACILITIES

Jeff Gruntmeir - Department of Comparative, Diagnostic, and Population Medicine, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Beth Nielsen** - Translational Genomics Research Institute (TGen), Flagstaff, AZ; **Haley Yaglom** - Translational Genomics Research Institute (TGen), Flagstaff, AZ; **Jessica Siegal-Willott** - United States Department of Agriculture, Animal Plant Health Inspection Service (USDA APHIS), Riverdale, MD; **Martha Keller** - United States Department of Agriculture, Animal Plant Health Inspection Service (USDA APHIS), Riverdale, MD; **Lucas Robertson** - United States Department of Agriculture, Animal Plant Health Inspection Service (USDA APHIS), Riverdale, MD; **Jim Wellehan** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Maureen Long** - Department of Comparative, Diagnostic, and Population Medicine, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Introduction: Transmission of the respiratory virus SARS-CoV-2 and its related variants to companion animals, farmed animals, zoo animals, and wildlife has been detected worldwide. In the US, SARS-CoV-2 has been detected in wildlife species in 32 states in a relatively limited number of species. Scientific knowledge is incomplete concerning current transmission and the reservoir potential of native wild mammal populations in the US states and territories. Licensed wildlife rehabilitators are uniquely positioned to provide opportunistic sampling for surveillance of pathogens in wildlife, especially SARS-CoV-2. The goal of this project is to enroll 50 wildlife rehabilitation facilities in the United States and Territories for testing wildlife for SARS-CoV-2 presented to these facilities over a two-year period.

Methods: This project is ongoing and following appropriate permissions and permitting for scientific collection from wildlife specific to each state, samples consisting of nasal and fecal swabs are collected and tested for SARS-CoV-2 nucleic acids and remnant blood for SARS-CoV-2 antibodies. Samples are tested by Translational Genomics Research Institute (TGen),

a third-party not for profit test facility, for molecular and antibody testing. All presumptive positive samples are then sent to the National Veterinary Services Lab for confirmation. In addition, a biosecurity survey is completed by each facility to gain understanding of biosafety procedures and the level of biosecurity maintained at facilities.

Results: Preliminary progress and results in the first six months of this project include scientific collection permissions or permitting in 22/50 states, contact with 101 facilities, resulting in enrollment of 17 wildlife rehabilitation facilities. Altogether, 333 samples collected from 131 individuals have been submitted from 20 species with 2/333 samples testing presumptive positive. A total of19 facilities have provided biosecurity assessments including two not participating in sample submission.

Conclusions: While facility enrollment, sample submission and testing are still ongoing, preliminary results suggest that exposure and active infections among US native wildlife entering state licensed wildlife rehabilitation facilities is limited. With accumulation of data from the biosecurity assessment, we will be able to provide a comprehensive understanding of the level of biosecurity across facilities which will aid in development of 'best practices' for prevention of spread of SARS-CoV-2 from handlers to wildlife and between animals undergoing rehabilitation.

23. AN ESSENTIAL ROLE OF HEPCIDIN IN DISSEMINATING CANDIDIASIS

Tanmay Arekar - Department of Medicine, College of Medicine, University of Florida; **Divya Katikaneni** - Department of Medicine, College of Medicine, University of Florida; **Nazli Khodayari** - Department of Medicine, College of Medicine, University of Florida; **Leena Al-Hraki** - Department of Medicine, College of Medicine, University of Florida; **Elizabeta Nemeth** - University of California, Los Angeles; **Alexander Drakesmith** - University of Oxford, Oxford, UK; **Michail Lionakis** - NIAID, NIH; **Borna Mehrad** - Department of Medicine, College of Medicine, University of Florida; **Yogesh Scindia** - Department of Medicine, College of Medicine, University of Florida

Introduction: Candida albicans (C. albicans) is a commensal fungus, and its transition from commensalism to pathogenicity remains the predominant cause of invasive candidiasis, with up to 40% mortality. While the risk of systemic candidiasis and clinical outcomes vary significantly, the host-related risk factors are not well-defined. For example, patients with chronic liver disease (CLD) are more susceptible to candidemia despite the absence of neutropenia.

Iron is an essential micronutrient for host physiology and a growth factor for C. albicans. Systemic iron metabolism is primarily regulated by the hepcidin-ferroportin axis. The liver is the primary source of hepcidin (encoded by the HAMP gene). Hepcidin binds to and triggers the internalization of ferroportin, the only known mammalian iron transporter. Inhibition of ferroportin mediated iron export, increases intracellular iron, reduces iron export into the plasma and is a primitive host defense strategy to counter microbial iron acquisition. CLD patients synthesize less hepcidin. Whether impaired hepcidin production influences the outcomes of disseminating candidiasis is unknown.

Method: Genetic and tamoxifen-inducible hepcidin knockout (Hamp-/-) and their wild-type (WT) littermates were infected with C. albicans SC5314. In separate experiments, PR-73 (synthetic mini hepcidin) was administered to a cohort of Hamp-/- mice. The outcomes of infection were evaluated after 3 and 6 days.

Results: Genetic and inducible hepcidin deficiency resulted in rapid iron overload in the kidney and liver. However, the fungal burden in the kidney was orders of magnitude higher than in the liver. Compared to WT mice, genetic and inducible Hamp-/- mice displayed increased renal fungal burden, more renal injury, and inflammation. On day 3, C. albicans was in yeast form in the kidneys of WT mice but had transformed into hyphae in the iron-rich renal tubular segments of Hamp-/- mice. This was associated with loss in the renal parenchyma and increased mortality. PR-73 treatment reduced renal fungal burden, hyphal transformation, and renal injury in Hamp-/- mice.

Conclusion: Our data identify hepcidin deficiency as a novel host susceptibility factor in C. albicans-induced renal failure and mortality. Hepcidin deficiency was associated with increased renal iron burden and accelerated hyphal transformation, indicative of increased virulence. Since PR-73 (mini hepcidin) mitigated C. albicans-induced pathology, our study identifies ferroportin as a druggable target to ameliorate outcomes of disseminating candidiasis. Our data lay the foundation for evaluating the efficacy of hepcidin analogs as possible therapeutics especially in liver disease patients to attenuate C. albicans-induced pathology.

24. CHARACTERIZATION OF VARIATION IN VIRULENCE-RELATED GENES OF PHYTOPHTHORA PALMIVORA ASSOCIATED WITH CACAO

Mariana Herrera Corzo - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Andrew Gitto** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Alina Puig** - USDA-ARS, Fort Detrick, MD; **Yeirme Yaneth Jaimes Suárez** - Centro de Investigación La Suiza, Corporación Colombiana de Investigación Agropecuaria (Agrosavia), Rionegro, Santander, Colombia; **Bryan Bailey** - USDA-ARS, Fort Detrick, MD; **Jeremy Brawner** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Erica Goss** - Emerging Pathogens Institute, University of Florida

Phytophthora palmivora is a pathogen with worldwide distribution that affects several tropical and subtropical hosts, including economically important crops such as papaya, durian, coconut, rubber, oil palm, and cacao. Understanding the diversity and evolution of this pathogen is essential for effective disease management strategies. The ability of P. palmivora to infect a wide range of plants suggests a broad set of strategies to overcome the host defenses, making the characterization of virulence/pathogenicity factors associated with this organism of great interest. As a first step towards describing these factors across the species, a collection of 57 cacao isolates from America were sequenced to determine the composition of effector genes and other putative virulence factors. A targeted sequence capture method, with probes designed from a whole genome reference sequence, was used to obtain high-depth sequencing of effectors. With these data, we examined variation in effector genes in isolates collected from cacao within and among geographic regions, elucidating profiles associated with subregions.

25. CHARACTERIZING LEISHMANIA DONOVANI INFECTION WITHIN HEPATIC STELLATE CELLS

Tia Monjure - Department of Biomedical Engineering, College of Engineering, University of Florida; **Joshua Ruley** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Javier Rosero** - College of Agricultural and Life Sciences, University of Florida; **Peter Kima** - College of Agricultural and Life Sciences, University of Florida; **Ana Maria Porras** - Department of Biomedical Engineering, College of Engineering, University of Florida

Visceral leishmaniasis (VL) is a neglected tropical disease spread by the bite of an infected sandfly with ~95 percent mortality rate of untreated cases. Currently, there are no VL vaccines, and the treatments are extremely toxic. This is partially due to a lack of understanding of the etiology of the disease. One symptom of VL is the enlargement of the liver. However, the changes in liver architecture post-infection are not well characterized and the mechanisms that drive liver enlargement have not been identified. We hypothesize that Leishmania infection leads to extensive remodeling of the extracellular matrix (ECM of the liver). Because hepatic stellate cells (HSCs) are the drivers of ECM production in the liver, this study focuses on examining the impact of Leishmania infection on HSC phenotype and ECM production. Using the LX-2 HSC cell line, our first objective was to assess whether HSCs can be infected with Leishmania donovani. Using immunocytochemistry, we observed that parasites can indeed infiltrate HSCs and may even differentiate into proliferative amastigotes after 24 hours. In parallel, we saw differences in alpha smooth muscle actin expression, a marker for hepatic stellate cell activation, 24 hours after infection. Ultimately, the HSCs perish significantly after 72 hours of infection, indicating that Leishmania donovani does infect and cause changes in HSC phenotype. The results of this analysis will shed light on the impact of Leishmania infection on HSC activation and ECM deposition. Thus, we will begin to uncover some of the mechanisms that drive uncontrolled tissue growth in this disease.

26. CRYPTOCOCCUS NEOFORMANS GLUCURONOXYLOMANNAN COMPROMISES MICROGLIAL CHEMOTAXIS VIA INHIBITION OF PURINERGIC RECEPTORS

Rafael Garcia - Department of Oral Biology, College of Dentistry, University of Florida; **Luis R. Martinez** - Department of Oral Biology, Emerging Pathogens Institute, College of Dentistry, University of Florida; **Claudia Charles-Nino** - Department of Oral Biology, College of Dentistry, University of Florida

Purinergic receptors (PR) are a class of cellular membrane receptors that respond to extracellular purines such as ATP and ADP, which may serve as an alternative mode of activation in immune cells. The ionotropic (P2X) and metabotropic (P2Y) PRs in microglia play a significant role in their activation, proliferation, and chemotaxis. Cryptococcus neoformans is an encapsulated fungus that causes life-threatening meningoencephalitis in individuals with AIDS. Given the limited data on the interactions of the fungus and microglia, we investigated the impact of glucuronoxylomannan (GXM), the main component of C. neoformans capsule, on P2X4 and P2Y12 receptors on microglia and phosphoinositol-3-kinase (PI3K) signaling pathway. We hypothesized that the GXM inhibits microglia's PR surface distribution and P13K signaling. To test this, microglia were pretreated with 10 μg/mL GXM for 4-hours at 37°C in 5% CO2, followed by 2-hours activation with ADP. Confocal microscopy and flow cytometry were used to measure microglial PR distribution upon GXM exposure, while immunoblot was used to assess the expression of PR and the components of the P13K pathway. Inhibitors NP-1815PX (P2X4) and Clopidogrel (P2Y12) were used as negative controls. Bacterial lipopolysaccharide, which activates PR's Pl3K signaling, was used as a positive control. Our results indicate that GXM inhibits the expression of P2X4 and P2Y12, which may have a detrimental role in microglial chemotaxis and infection control due to inactivation of the PI3K signaling cascade. Future experiments will use mouse models of cryptococcal infection to understand the effects of Cn GXM on microglia PR in brain infection control.

27. CRYPTOCOCCUS NEOFORMANS SHOWS INCREASED PATHOGENESIS IN MICE AFTER INTERACTIONS WITH STAPHYLOCOCCUS AUREUS

Claudia Charles-Nino - Department of Oral Biology, College of Dentistry, University of Florida; **Cesar Pedroza Roldan** - Universidad de Guadalajara; **Ernesto Becerra-Jimenez** - Universidad de Guadalajara; **Janeth Anaya-Ambriz** - Universidad de Guadalajara; **Rafael Cortez Zarate** - Universidad de Guadalajara; **Gustavo Freitas** - Universidade Federal de Minas Gerais; **Daniel De Assis Santos** - Daniel De Assis Santos; **Glauber Ribeiro de Sousa Araujo** - Universidade Federal do Rio de Janeiro; **Susana Frases** - Universidade Federal do Rio de Janeiro; **Luis R. Martinez** - Department of Oral Biology, Center for Immunology and Transplantation, Emerging Pathogens Institute, College of Dentistry, University of Florida

Introduction: Cryptococcus neoformans (Cn) is an opportunistic, neurotropic, and encapsulated fungus that causes more than 150,000 worldwide cases of cryptococcal meningitis in people living with HIV/AIDS each year. Methicillin-resistant Staphylococcus aureus (Sa) colonization and infections have increased in the last years and an 18-fold higher rate are reported in people living with HIV/AIDS. Polymicrobial interactions trigger synergistic or antagonistic effects in the virulence of microbes. The interaction between Cn and Sa is possible since both can colonize and infect the skin, lungs, and brain. Furthermore, it is possible that environmental Cn-Sa interaction can generate virulent strains transmissible to humans. We hypothesized increased Cn pathogenesis in mice after interaction with Sa.

Method: Cn strain H99 and Sa strain NR45992 were used in vitro and in a pulmonary co-infection mouse model. Growth curves, Cn capsule measurements, proteomic analysis of the secretome, and scanning electron microscopy were performed during Cn-Sa interactions in vitro. Balb/c mice were intratracheally infected with 105 CFU of Cn, Sa, or Cn and Sa and survivability studies were performed. To assess disease progression, fungal load and capsule size determinations, histopathology, and cytokine measurements were evaluated in lung and brain tissue.

Results: Cn capsule size was significantly reduced in co-culture with Sa conditions compared to single culture after 6-, 24-, and 48-hours. Unique proteins involved in Cn capsule synthesis were identified in the secretome of 24-h co-cultures. In contrast, superantigens, enterotoxins, and antibiotic resistance proteins were produced by Sa after interactions with Cn. Co-infected mice showed high bacterial and fungal burden in brain tissue at 3- and 7-days post-infection. However, only a significantly high fungal burden was found in the lungs. Surprisingly, Cn capsule size was significantly enlarged in co-infected mice at 3- and 7-days post-infection.

Conclusions: Our findings suggest that polymicrobial interactions influence the regulation and production of virulence factors by individual microorganisms, important elements needed for their survival and successful colonization of the human host.

28. EPIDEMIOLOGICAL CHARACTERISTICS AND OUTCOMES AMONG PATIENTS DIAGNOSED WITH NEUROCYSTICERCOSIS (NCC) AT UF HEALTH SHANDS HOSPITAL: A 30 YEAR RETROSPECTIVE ANALYSIS

Maggie Zawoy - College of Medicine, University of Florida; **Waverly Leonard** - College of Medicine, University of Florida; **Norman L. Beatty** - Department of Medicine, Emerging Pathogens Institute, University of Florida

Introduction: Neurocysticercosis (NCC) is a parasitic disease of the central nervous system caused by the pork tapeworm, Taenia solium. NCC is the leading cause of acquired seizure disorder worldwide. In the United States (US) this disease is seen most among those who have immigrated from endemic regions within the tropics.

Methods: A retrospective analysis was conducted from hospital records between 6/1/1993 until 6/1/2023 using specific ICD-9 and ICD-10 codes for NCC. Inclusion criteria for this study included those presenting to UF Health Shands Hospital (Gainesville, Florida) with asymptomatic or symptomatic NCC. Diagnostic criteria for NCC consisted of radiologic features , serologic evidence T. solium infection or pathological tissue confirmation. 34 patients met inclusion criteria. We collected

epidemiological and demographic characteristics, hospitalization course, and clinical outcomes for each case.

Results: Among the 34 patients, 59% identified as male and 41% female. Majority (n=26/34) had immigrated from outside the US and 46% of those were from Mexico (n=12/26). Other regions included, Guatemala (n=4), Haiti (n=2), El Salvador (n=2), Dominican Republic, Honduras, India, Mozambique, Nepal, and Peru. 65% Hispanic, 32% non-Hispanic, 3% unspecified. The majority were between the ages 21-50 (68%) while 18% were between 51-70 and the remaining 15% were between 0-20. 41% were uninsured, 21% insured, 38% unspecified. Language preferred for medical correspondence included 47% Spanish, 38% English, 6% other, 9% unspecified. Selected hospitalization outcomes were also collected. 97% of patients reported symptoms (seizures, focal neurologic deficits, headaches, hydrocephalus, nuchal rigidity, psychiatric disturbances, and altered mental status) prior to receiving an NCC diagnosis. 72% of patients presented to at least one healthcare facility for similar symptoms prior to diagnosis of NCC. Patients presented with headaches (61%) and seizures (50%). Interestingly, 24% presented with CNS deficits and 26% with altered mental status. 52% were diagnosed with another condition prior to their diagnosis of NCC. Extraparenchymal cyst burden significantly correlated with longer hospital stay. Many patients either received medications for inactive infection or did not receive proper medication. 21% of patients were readmitted at least once for NCC symptoms or complications. 18% of patients stayed in the hospital for more than 10 days.

Conclusions: NCC is a neglected tropical disease which is often overlooked in those who have immigrated from endemic regions. Majority of those living with NCC in Florida were from Mexico but increased awareness of NCC among clinicians can help with timely management and treatment.

29. IL-6 DEFICIENCY FACILITATES CRYPTOCOCCUS NEOFORMANS COLONIZATION OF THE CENTRAL NERVOUS SYSTEM

Marta Reguera Gomez - Department of Oral Biology, College of Dentistry, University of Florida; **Melissa Munzen** - Department of Oral Biology, College of Dentistry, University of Florida; **Claudia Charles-Nino** - Department of Oral Biology, College of Dentistry, University of Florida; **Luis R. Martinez** - Department of Oral Biology, College of Dentistry, University of Florida

Introduction: Cryptococcus neoformans is an opportunistic encapsulated fungus that causes life-threatening meningoencephalitis in immunosuppressed individuals. CD4 T cells, are a crucial component of the cell-mediated immunity to C. neoformans. Th1-type responses are characterized by the production of IL-2, IL-12, IFN-γ and TNF-α. C. neoformans also induces secretion of other pro-inflammatory cytokines, including IL-6 from innate immune cells which together with IL-1β, induces the development of T-helper Th17 cells in the presence of IL-23.

Method: The impact of IL-6 on C. neoformans systemic infection was investigated in vivo and in vitro. We infected intravenously wild-type C57BL/6, IL-6 knock-out (IL-6−/−) and IL-6−/− treated with recombinant IL-6 (rIL-6; 40 pg/g) mice to assess its involvement on the survival and central nervous system (CNS) pathophysiology.

Results: IL-6−/− mice showed significantly higher mortality than wild-type and IL-6−/− + rIL-6 mice. IL-6−/− mice showed the highest fungal burden in blood and brain. Wild-type and IL-6−/− + rIL-6 microglia showed predominantly an activated/phagocytic phenotype while IL-6−/− microglia were mainly dystrophic. Since IL-6 production and effect are directly related to these resident CNS immune cells, we observed an increased in phagocytosis and killing efficacy in microglial cells cultured with rIL-6 in vitro. Astrocytes showed lower numbers and less hypertrophy of processes in the IL-6−/− mice brains. Finally, we analyzed the effect of IL-6 on C. neoformans capsule size showing an increase in capsule growth after 48h incubation with rIL-6 in vitro.

Conclusions: Our findings suggest that IL-6 plays a critical role in the host CNS defense against C. neoformans. Identifying novel mechanisms by which C. neoformans alters brain immunity will provide new insights into this neuropathology.

30. INHIBITION OF RHOA PREVENTS CRYPTOCOCCUS NEOFORMANS CAPSULE GLUCURONOXYLOMANNAN-STIMULATED BRAIN ENDOTHELIAL CELL INTERACTION BREAKDOWN.

Melissa Munzen - Department of Oral Biology, College of Dentistry, University of Florida; **Cristian Mathew** - Department of Biology, Hofstra University; **Vanessa Enriquez** - Department of Oral Biology, College of Dentistry, University of Florida; **Amanjeet Minhas** - Department of Biology, Hofstra University; **Claudia Charles-Nino** - Department of Oral Biology, College of Dentistry, University of Florida; **Durvinand Saytoo** - Department of Biology, Hofstra University; **Michael R. Dores** - Department of Biology, Hofstra University; **Luis R. Martinez** - Department of Oral Biology, Center for Immunology and Transplantation, Emerging Pathogens Institute, College of Dentistry, University of Florida

Cryptococcus neoformans is an opportunistic fungal pathogen capable of causing severe CNS disease in immunocompromised individuals. Invasion of the brain parenchyma requires fungal traversal of the selectively impermeable blood-brain barrier (BBB). C. neoformans harbors diverse mechanisms by which it interacts with the BBB endothelium layer and can invade through transcellularly through internalization by endothelial cells, paracellularly by tight junction disruption between endothelial cells, and concealed from immune recognition within host cells as cargo. In this study, we describe a mechanism by which C. neoformans alters the brain endothelium by activating small GTPase RhoA, which facilitates reorganization of the actin cytoskeleton and tight junction modulation to regulate endothelial barrier permeability. We confirm the role of the fungal capsule polysaccharide GXM in initiating these alterations, which are inhibited by targeting RhoA. Furthermore, we reveal a therapeutic benefit of RhoA inhibition by CCG-1423 in vivo. By restoring the integrity of the BBB endothelium through RhoA inhibition, prolonged survival and reduced fungal burden is observed in a murine model of disseminated

cryptococcosis, supporting the therapeutic potential of this compound in the context of cryptococcal infection. This study examines the complex virulence of C. neoformans in establishing CNS disease and describes cellular components of the brain endothelium that may serve as molecular targets for future antifungal therapies to alleviate the burden of life-threatening cryptococcal CNS infection.

31. MULTIPLE ROUTES OF HORIZONTAL PATHOGEN TRANSMISSION IN A SOCIAL SPIDER

Steven T. Cassidy - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Abigail Pope** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Nolan Missigman** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Kara Taylor** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Martha Haufiku** - University of Namibia; **Tresia Kavili** - University of Namibia; **Seth Eiseb** - University of Namibia; **Carl N. Keiser** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Social interactions are a driving force behind infectious disease outbreaks in animal societies. As groups become more complex, they run the risk of introducing more routes of potential pathogen exposure and transmission. For example, social animals that build and live inside nests, burrows, or retreats can increase the number of potential direct and indirect transmission events inside the nest. Highly complex societies like eusocial ants have social immune defenses against disease outbreaks within the nest, though few studies have compared multiple routes of pathogen exposure in other nest-building social animals. We exposed groups of social spiders (Stegodyphus dumicola) to a generalist entomopathogenic fungus (Metarhizium robertsii) using three modes of exposure: directly onto a group-mate, mechanically vectored by a living prey item, and environmental exposure via shared substrate. We compared spider mortality in all three exposure treatments as well as pathogen-free control groups. Daily we also recorded whether each spider inside or outside their shared nest to test whether pathogen

exposure route, and by extension, disease outbreak severity, affected spiders' propensity to aggregate in their nest during daylight hours. We found that different routes of pathogen transmission greatly affected spider mortality, with colonies experiencing direct exposure from a nestmate experiencing the most rapid mortality. Individuals from groups with a directly exposed spider were also more likely to be observed outside their nest compared to all other treatments. These data show that route of pathogen transmission affects not only the severity of disease outbreaks but can alter the behavior of nest-building groups.

32. NECROPTOSIS ASSOCIATES WITH INFECTION SEVERITY AND BIRTH OUTCOMES IN PLACENTAL MALARIA

Demba Sarr - Department of Infectious Disease; **Brittany N. Russ** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Julie Moore** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Placental damage and dysfunction are prominent in malaria infection and associate with poor birth outcomes. Necrotic death and shedding of villous trophoblast may be a critical factor that influences placental function in this context. Our objective was to investigate whether the syncytiotrophoblast undergoes necroptosis during placental malaria. Primary human trophoblast (PHT), villus explants and placenta from women and mice exposed to malaria were used to assess markers of regulated necrosis by RT-qPCR, western blot and immunostaining. Two markers for necroptosis, receptor interacting protein kinase 3 (RIP3) and phosphorylated mixed lineage kinase domain-like protein (pMLKL), were significantly higher in human placenta with malaria infection, and RIP3 was inversely correlated with infant birth weight. Furthermore, in vitro exposure of syncytialized primary human trophoblast (PHT) to the malaria toxin, hemozoin, induced elevated transcripts for RIP1 (RIPK1) and RIP3 (RIPK3), which are required for propagation of necroptosis. However, in vitro activation of human primary cytotrophoblast and syncytiotrophoblast as well as primary placental villous explants through the death receptor pathway (treatment with antibodies to tumor necrosis

factor, plus cycloheximide and carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone (Z-VAD)), which in other epithelial cell types drives necroptosis, did not induce this cell death pathway. While expression and redistribution of RIP1 within the villus was observed, no RIP3 or pMLKL was detected in cells or explants. Instead, trophoblast apoptosis was observed with this treatment. Only treatment conditions that included the caspase inhibitor Z-VAD were able to restore PHT viability to control levels. Thus, under the in vitro conditions used in this study, PHT is resistant to necroptosis, yet, in vivo, this cell death pathway could play an important role in the pathogenesis of placental malaria. Regulation of cell death pathways in the syncytiotrophoblast requires further investigation to establish critical triggers that can precipitate placental loss of function and pregnancy compromise.

33. ROBUST ASTROCYTE ACTIVATION IN RESPONSE TO CRYPTOCOCCUS NEOFORMANS BRAIN INFECTION IN MICE

Adrian Requejo - Department of Neuroscience, College of Medicine, University of Florida; **Karina Alviña** - Department of Neuroscience, College of Medicine, University of Florida; **Luis R. Martinez** - Department of Oral Biology, College of Dentistry, University of Florida; **Melissa Munzen** - Department of Oral Biology, College of Dentistry, University of Florida

The encapsulated fungus Cryptococcus neoformans (Cn) is the causative agent of cryptococcosis. Despite aggressive antifungal treatment, immunocompromised patients are highly susceptible to develop and die from cryptococcal meningoencephalitis (CME). The polysaccharide capsule of Cn greatly affects the host immunity and its main component, glucuronoxylomannan (GXM), enhances fungal brain infection. Cn enters the brain via cerebral capillaries and crosses the blood brain barrier (BBB) utilizing diverse mechanisms. However, there are important knowledge gaps regarding underlying brain cell responses and the consequences of this fungal infection for brain function. Astrocytic activation in response to various central nervous system insults is fundamental for brain homeostasis. Reactive astrocytes are associated with destructive cryptococcal brain lesions and high accumulation of GXM in tissue from

patients with CME. Therefore, we hypothesized that as Cn colonizes brain tissue during infection, there is a close interaction between Cn and astrocytes that modulates distinct intracellular pathways in both cells. To test this hypothesis, we first used a mouse model of Cn infection and quantified the number of reactive astrocytes using immunohistochemical techniques. Compared to uninfected controls, infected mice had significantly more activated astrocytes surrounding Cn lesions. In addition, using an in vitro model of astrocytic cells exposed to GXM, we observed a significant increase in the expression glutamatergic transporters and receptor subunits. Similarly, we found that the Cn capsule enlarges, and genes involved in capsule formation are increased in Cn cells exposed to astrocytes. We are currently expanding these findings to fully determine the molecular and cellular mechanisms underlying the astrocytic response during CME. Identifying mechanisms by which Cn interacts with astrocytes will provide novel insights into the neurotropism of this deadly infection. This information may also offer new avenues for combating CME, a disease that kills ~200,000 people per year worldwide.

34. THE EFFECTS OF INFECTION BY A NON-TROPHICALLY TRANSMITTED PARASITE ON SNAIL HOST BEHAVIOR

Eric Trotman - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Jesus Rodriguez-Riverol** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Ila Gapinski** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida; **Carl N. Keiser** - Department of Biology, College of Liberal Arts and Sciences, University of Florida

Introduction: Many parasites have been documented altering the behavior of their hosts, especially in those with complex life cycles where transmission occurs via predation (i.e., trophic transmission). Less is known about how parasites alter hosts behavior when intermediate life stages are motile, such as trematode cercariae which are abundant and diverse across many aquatic ecosystems. Here, we aimed to study the behavior of infected and uninfected intermediate host snails across different ecological contexts. Planorabella trivolvis is a freshwater air-breathing snail, known to be an intermediate host for many parasites but is predominantly associated with Echinostoma spp. trematodes. Across their life cycle, these multi-host parasites infect a wide range of taxa from freshwater snails to amphibians, fish, and wading birds, and impose public health concerns to human populations.

Goals: (1) assess infection prevalence and intensity across four P. trivolvis populations in Gainesville FL, (2) quantify differences in the behavior of infected and uninfected snails, and (3) measure snail movement in the presence of predation cues.

Methods: We checked field-caught snails for infection with Echinostoma trematodes using environmental chambers to stimulate cercarial shedding and tested snails' righting behavior and phototaxis in the laboratory.

Results: Infected snails were slower to right themselves after being placed on their side and were slower to emerge from a retreat into the light compared to uninfected snails. We then measured snails' responses

to varying levels of predation risk cues: control with no predator cues, exposure to a caged crayfish, and exposure to a crayfish plus a conspecific alarm cue. Infected snails moved further away under all conditions compared to uninfected snails.

Discussion: These data show that, although it is uncertain if these trematode parasites manipulate snail host behavior, infected snails exhibit context-dependent altered behaviors. Freshwater snails are intermediate hosts for many parasites and studying the behavior of infected snails will help us understand the impact of multi-host parasites on ecosystems and can inform the conservation efforts of imperiled species which may be potential hosts.

35. TRACKING THE RECRUITMENT OF ESCRT TO THE LEISHMANIA DONOVANI PARASITOPHOROUS VACUOLE

Javier Rosero - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Peter Kima** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Leishmania are flagellated protozoa in the Kinetoplastid family that have been classified by the WHO as the causative agents of a neglected tropical disease. Leishmania donovani (Ld) is a species of this intracellular pathogen that is known to cause potentially fatal visceral leishmaniasis in vertebrate hosts. Ld is phagocytosed by macrophages of the host, wherein they undergo a morphological change from the promastigote to the amastigote form. Ld live in parasitophorous vacuoles (PV) that fuse extensively with lysosomes. PVs have been shown to acquire components of the secretory and endocytic pathways for nutrition acquisition and pathogenesis. Like other intracellular organisms, Leishmania may deploy macromolecules that interfere with or engage processes in the host cell. An important molecular complex in the endocytic pathway is the endosomal sorting complex required for transport (ESCRT) which is comprised of 5 sub-units. This protein complex participates in several biological activities including fission and scission of endosomal vesicles.

Interestingly, individual subunits of ESCRT can be recruited independently to perform various tasks such as the repair of the plasma membrane, correction of defects in the nuclear envelope, abscission of the midbody in cells undergoing cytokinesis, and maintenance of the endomembrane system such as repair of the membrane of lysosomes, which are prone to rupture. The role of the ESCRT machinery in Leishmania and its vacuolar membrane is not known. To initiate studies on the ESCRT machinery in Leishmania infections, we took advantage of ESCRT fluorescent tagged proteins that were available to us. Specifically, we acquired plasmid constructs of the ESCRT-I member, Tumor Susceptibility Gene 101 (TSG101), and ESCRT-III members, Charged Multivesicular Protein 2B/4B (CHMP2B/4B) and the Vacuolar Protein Sorting 4A (VPS4A). Macrophages were transfected with these plasmids and infected with Ld. Subsequent immunofluorescent analyses of the distribution of these molecules in infected cells have revealed that members of the ESCRT machinery are recruited to Ld PVs to varying degrees. Ongoing studies will extend these analyses and determine the functional roles of the ESCRT machinery in Leishmania infections.

36. A NOVEL MURINE MODEL FOR INVESTIGATING INFLAMMATORY AND COAGULATION MECHANISMS IN MALARIA-ASSOCIATED RESPIRATORY DISTRESS SYNDROME

Nicole M. Nazario-Maldonado - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Margaret Taylor** - Department of Microbiology and Cell Science, College of Liberal Arts and Sciences, University of Florida; **Julie Moore** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida

Introduction: Malaria is a bloodborne parasitic disease transmitted by mosquitoes and presents a global health challenge. Severe cases of malaria often lead to Malaria-associated acute respiratory distress syndrome (MA-ARDS), marked by pulmonary complications such as edema, inflammation, hemorrhages, and alveolar damage. This study will address the gaps in understanding MA-ARDS pathogenesis, focusing on pulmonary lesions and vascular leakage, with a specific emphasis on severe pathology in pregnant women.

Methods: To elucidate the underlying pathogenic mechanisms of lung malaria, we propose the development of an experimental murine model (pregnant and non-pregnant). This model aims to investigate the role of inflammation and coagulation in MA-ARDS, characterized risk factors from sexual dimorphism and pregnancy, while emphasizing the contributions of innate cells and parasite byproducts to vascular leakage and hemorrhages. To achieve this, an in vivo murine lacking the expression of tissue factor, the primary initiator of blood coagulation, will be employed.

Expected results: We expect that modifying tissue factor expression will suppress the inflammatory response and mitigate endothelial disruption, resulting in reduced vascular leakage in the lung.

Conclusion: This study seeks to provide a comprehensive understanding of the pathology of malaria parasites in the lungs and aims to offer a basis for developing therapeutic treatments against severe malaria. By establishing a suitable experimental murine model, our research endeavors to contribute valuable insights into the complex interplay between inflammation, coagulation, and vascular integrity during MA-ARDS, ultimately advancing our knowledge and fostering the development of targeted therapeutic interventions.

37. AEDES AEGYPTI LARVAL HABITAT DETRITUS, NUTRIENTS, AND COMPETITION ALONG AN URBAN GRADIENT IN PUERTO RICO: INDICATORS FOR DENGUE VIRUS VECTOR COMPETENCE?

Limarie J. Reyes-Torres - Department of Biological Sciences, University of Southern Mississippi; **Barry Alto** - Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Donald Yee** - Department of Biological Sciences, University of Southern Mississippi

Introduction: Aedes aegypti is the primary vector of emerging arboviruses causing serious human illnesses including dengue virus, Zika, and chikungunya. The mosquito species population dynamics are related to urbanization and human density as larvae develop in human-made habitats e.g., discarded containers, and adults blood feed on humans. Changes in the urban landscape could influence detritus inputs, nutrient availability, and mosquito species competition in larval habitats. These effects could impact Ae. aegypti density, biomass, and other aspects across life stages, generations, and its vector competence for dengue virus.

Methods: Fifty-four containers were sampled across an urban gradient in the San Juan Metropolitan Area, Puerto Rico during June-August of 2021 and 2022. A 1.52m radius survey from each container was conducted to determine vegetation species α diversity. A 7.62m transect was walked from each container while counting houses and green spaces, as a fine scale, visual urban variable. Nutrient and isotopes analyses (i.e., %Carbon %Nitrogen, Carbon/Nitrogen, δ^{15}N, and δ^{1}3 C) were performed on

container water suspended particulate organic matter (SPOM), detritus, and larvae. A PCA was performed with 26 container and urban variables. Multivariate multiple regressions were conducted with principal components and Ae. aegypti dependent variables.

Results: Aedes aegypti dominated containers across all the urban gradient. Co-occurrence with its competitor Ae. mediovittatus (Caribbean treehole mosquito), was observed in 25.9% of containers. Urban density influenced the number of houses, number of green spaces, and vegetation α diversity near containers. Detritus inputs and nutrients isotopes in containers were also influenced by urban density. The PCA showed five principal components that explained 71.2% of variance in the data. Significant variables in the principal components included nutrients in competitor larvae, SPOM, and detritus, competitor biomass and density, detritus density, vegetation α diversity, house abandonment, and urban density. Ae. aegypti larval density, biomass, CN, $\delta^{15}N$, and $\delta^{1}3$ C were related to the main principal components.

Conclusion: This research showed the relationships between urbanization, larval container environment, and Ae. aegypti. Understanding how the urban gradient influences the conditions where Ae. aegypti develops and its life history is important as they can determine the potential of this species to spread arboviruses. The results of this research have relevance to vector ecology, vector control strategies, arbovirus diseases, and public health.

38. ANALYSIS OF THE RELATIONSHIP BETWEEN SOFT TICKS (ORNITHODOROS TURICATA) AND WILD PIGS (SUS SCROFA) IN FLORIDA: IMPLICATIONS FOR AFRICAN SWINE FEVER VIRUS INTRODUCTION TO THE UNITED STATES

Nicholas Canino - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Sebastian Botero-Cañola** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carson Torhorst** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Lorenza Beati** - US National Tick Collection, Institute for Coastal Plain Science, Georgia Southern University; **Kathleen O'Hara** - Center for Epidemiology and Animal Health (CEAH), Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS), USDA; **Angela James** - Center for Epidemiology and Animal Health (CEAH), Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS), USDA; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

The ongoing global panzootic of African Swine Fever (ASF) has highlighted the need for a comprehensive plan for the prevention and response to this disease in the United States. As a proactive measure, the United States Department of Agriculture released a Response Plan that outlined a thorough overview of how the outbreak would be handled. One of the pre-outbreak measures proposed is the assessment of the transmission risk associated with soft ticks. This assessment is of special interest in the state of Florida, which harbors a large population of feral swine overlapping the distribution of the soft tick Ornithodoros turicata, a proven vector of ASF. In this project, our goal was to support this risk assessment by first developing a standardized protocol for surveying ticks. Then, we used it to conduct statewide sampling and determine their feeding relationship to wild pigs through bloodmeal analysis. We sampled 102 sites across Florida, and at each site we collected soft ticks from five

gopher tortoise burrows, their preferred microhabitat in the state. Collected soft ticks were identified to species using morphological features and DNA extraction was performed on a maximum of 10 pools of 5 ticks from each burrow. Tick-swine interaction was assessed using qPCR with a swine-specific primer and analysis was replicated three times. If any of the three qPCR runs of any of the pools returned positive for swine DNA, then that site was considered positive for tick-swine interaction. In total, we collected 3,093 soft ticks from 57 of the 102 sites (55.9%), with all the soft ticks identified as O. turicata. We analyzed 536 pools using the qPCR protocol, from which 10 pools (1.9%) resulted positive for swine DNA. Pig activity within 5 meters of the burrow, a proxy for potential tick-swine interactions, was also reported at 4 of the sites. Considering that these soft ticks are strongly associated with gopher tortoise burrows, the detection of any relationship with pigs is biologically significant to the transmission of a pathogen as virulent as ASF. These results suggest that O. turicata could play a role in the transmission of ASF and highlights the need to further quantify the potential risk of sylvatic maintenance of the disease cycle posed by this species. More research is needed to both further uncover the relationship between these ticks and wild pigs and to understand which areas have the greatest transmission risk for ASF.

39. ANALYZING THE IMMUNITY AND ANTIGENIC CHANGES IN DENGUE OVER TIME

Kellie Goodnight - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Gabrielle Johnson** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Luke Trimmer-Smith** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Leah Katzelnick** - NIH; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Dengue is a Flaviviridae virus transmitted by mosquitos that is known for causing disease in humans. Recent work by our group has found that dengue viruses vary antigenically, with infection with one virus providing heterogenous protection to other dengue viruses (Science, 2021).

Using blood samples from African Green Monkeys, we found that viruses collected in Bangkok, Thailand collected over a 20-year period were antigenically growing more distant from each other over time. Antigenic variability of dengue viruses (DENV) may lead to variation in individual risk of infection and disease depending upon the antigenic relationship of circulating viruses and the viruses that individuals have been exposed to previously. In our present work, we aim to characterize the antigenic relationship of viruses using the neutralization response of human sera in order to validate the information from the African Green Monkey map and confirm that DENV are growing more distant from each other as characterized by human immune responses.

The goal of this study is to characterize the ability of a panel of sera collected from individuals that have experienced dengue infections to neutralize sixteen distinct dengue viruses and determine if the relative neutralization response is associated with antigenic relationships of viruses described by an existing antigenic map of dengue viruses created using sera from African Green Monkeys (AGM). Blood samples have been collected from individuals living in Thailand for which DENV infection

history is observed and well characterized. Using dengue viruses systematically sampled from a viral archive ranging from 1975 to 2015, we characterized neutralizing antibody responses to viruses collected during individual's life spans and viruses from future and past time periods.

Viruses were selected from ~4000 sequenced viruses from our viral archive. We used the Plaque Reduction Neutralization Test (PRNT) to characterize the immune responses of forty-four sera samples from individuals who have had detected DENV detected during longitudinal follow-up from 1998-2002. We used PRNT responses to build antibody landscapes characterizing individuals' response to multiple viruses whose antigenic relationship has been characterized using AGM based antigenic maps.

Understanding how dengue immune responses to a diverse set of viruses that individuals may be exposed to in their lifetime are formulated is critically in comparing immunity derived from infection and comparing it to responses mounted in response to vaccines. Antibody landscapes are also useful in characterizing the ecological pressures that DENV may face in attempting to evade or take advantage of pre-existing immunity.

40. ASSESSING SPATIAL PATTERNS OF DENGUE INCIDENCE IN NEPAL FOR 2022: A LOCAL INDICATOR OF SPATIAL ASSOCIATION (LISA) APPROACH

Simrik Bhandari - Department of Geography, Emerging Pathogens Institute, 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; **Sadie Ryan** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: Dengue fever, first officially reported in Nepal in 2004, is a significant public health problem, now considered endemic in the country. In 2022, Nepal experienced a record number of cases, an alarming total of 54,784 in a population of 29 million. The most recent prior peak was in 2019, with 17,992 cases reported, followed by a sharp drop to 530 and 540 cases in 2020 and 2021, respectively. Initially confined to the lower plains of Nepal, Dengue fever has now spread to higher elevations. To inform targeted and effective interventions like mosquito control programs and public health education, spatial analyses can describe the patterns of disease, facilitating efficient resource allocation in high-risk areas. Here, we mapped the distribution of dengue fever incidence in Nepal for the year 2022 and identified spatial heterogeneity in incidence across the country.

Methods: The 2022 dengue case data reports were obtained from Nepal's Ministry of Health and Population, along with 2021 census data from the National Statistics Office. Choropleth maps were created in ArcGIS Pro to visualize population, dengue cases, and peak dengue months. Dengue incidence rate (per 100,000 people) was calculated by district (n=77). To test for significant clusters and outliers in dengue distribution, Local Moran's I was implemented in GeoDa software, to identify areas with high and low disease incidence.

Results: Dengue cases were reported in all 77 districts of Nepal in 2022. The highest case numbers were reported in August, September, and October. The Moran's I analysis identified 6 hotspots representing

clusters of districts experiencing statistically higher dengue cases (High-High), and 10 coldspots representing clusters of districts experiencing statistically lower dengue cases. A High-Low outlier was detected in Dang district, indicating significantly higher dengue cases compared to its immediate neighbors.

Conclusions: Identifying dengue hotspots, cold spots, and outliers can enhance targeted intervention strategies for vector-borne disease management, improving effectiveness and cost-efficiency. This study identified six hotspots of dengue activity in the 2022 outbreak in Nepal, indicating potential areas to target and plan for monitoring in future dengue seasons. In addition to hotspots, coldspots might identify areas where ongoing interventions are already underway and successful, warranting further examination to inform planning. This study provides a baseline tool for future examination of spatial patterns of dengue in Nepal. Further investigation can incorporate additional variables, such as climatic conditions, socio-economic status, and land use patterns, to gain a more detailed understanding of dengue transmission.

41. ASSESSING THE VALUE AND RETENTION OF KNOWLEDGE FROM AN ONLINE TICK IDENTIFICATION AND TICK-BORNE DISEASE MANAGEMENT COURSE FOR THE SOUTHEASTERN UNITED STATES

Catherine A. Lippi - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Holly D. Gaff** - Department of Biology, Old Dominion University; **Alexis L. White** - Department of Biological Sciences, University of Rhode Island; **Sadie Ryan** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Background: Tick-borne diseases are a growing public health threat in the United States. Despite the prevalence and rising burden of tick-borne diseases, there are major gaps in baseline knowledge and surveillance efforts for tick vectors, even among vector control districts and public health agencies. To address this issue, an online tick training course (OTTC) was developed through the Southeastern Center of Excellence in

Vector-Borne Diseases (SECOEVBD) to provide a comprehensive knowledge base on ticks, tick-borne diseases, and their management.

Methods: The OTTC consisted of training modules covering topics including tick biology, tick identification, tick-borne diseases, and public health, safety, and surveillance. The course was largely promoted to vector control specialists and public health employees throughout the southeastern US. We collected assessment and survey data on participants to gauge learning outcomes, perceptions of the utility of knowledge gained, and barriers and facilitators to applying the knowledge in the field.

Results: The OTTC was successful in increasing participants' baseline knowledge across all course subject areas, with the average score on assessment increasing from 62.6% (pre-course) to 86.7% (post-course). The majority of participants (63.6%) indicated that they would definitely use information from the course in their work. The main facilitator (70.4%) for applying knowledge was having opportunities at work, such as an existing tick surveillance program.

Conclusions: Overall, this OTTC demonstrated capacity to improve knowledge in a necessary and underserved public health field, and a majority of participants use or plan to use the information in their work. The geographic reach of this online resource was much larger than simply for the Southeastern region for which it was designed, suggesting a much broader need for this resource. Understanding the utility and penetrance of training programs such as these is important for refining materials, and assessing optimal targets for training.

42. CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 – 2023.

Amira Richardson - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Michael Lyons** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Merrie Urban** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Lillian Maxwell** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **An-Chi Cheng** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, College of Veterinary Medicine, University of Florida; **Kristen Wilson** - Department of Wildlife Ecology and Conservation, College of Veterinary Medicine, University of Florida; **Rayann Dorleans** - Department of Wildlife Ecology and Conservation, College of Veterinary Medicine, University of Florida; **John Lednicky** - Department of Environmental and Global Health, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

White-tailed deer farming is an important economic sector in Florida. However, it faces challenges from bacterial infections and viral hemorrhagic diseases (Epizootic Hemorrhagic Disease and Bluetongue Virus), which cause significant mortality among fawns and yearlings. To address this issue, the University of Florida's Cervidae Health Research Initiative (CHeRI) provides a free diagnostic service to Florida deer farmers to identify the causes of death in deer. From 2017 to 2023, diagnostic testing on 780 farmed white-tailed deer involved analyzing tissue samples using RT-qPCR, microbial culture, histopathology analysis, and parasite identification. Data analysis revealed substantial bacterial and viral disease prevalence variations among different age groups. For 253 deceased deer aged 1-90 days, 41.7% succumbed to bacterial infection and 21.9% to viral hemorrhagic disease. In the 4-12 months age group (242 animals), 66.9% died from hemorrhagic disease viruses and

16.9% from bacterial infection. Among 251 animals aged 13 months and older, 46.2% died from hemorrhagic disease viruses and 33.9% from bacterial infection. Overall, viral hemorrhagic diseases and bacterial infections constituted 76% of deaths in farmed white-tailed deer aged one day to 13 months and older. Analysis unveiled a seasonal pattern, with peak mortality in late summer to early fall, mainly due to viral hemorrhagic diseases. In 2023, Epizootic Hemorrhagic Disease Virus (EHDV)-positive cases attributed to EHDV serotypes 1, 2, and 6 were noted, with EHDV-1 having a significant outbreak in 2019 and 2023. Additionally, to understand its role in deer mortality, exploratory testing has been carried out for Mule Deerpox Virus (MDPV) in suspected necropsies. These insights offer valuable information on the prevalence and seasonal dynamics of pathogens affecting farmed white-tailed deer, guiding the development of effective management practices and treatment strategies.

43. CHARACTERIZATION OF PRO-VIRAL PROTEINS IN SECRETED SALIVA OF VARROA DESTRUCTOR

Sanghyeon Kim - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Arthropod saliva is a complex mixture of peptidic and non-peptidic molecules that facilitate feeding and serves as a medium for pathogen transmission. However, saliva is not simply a medium as some pathogens have been shown to exploit saliva-induced modulation of host responses that promote their transmission and infection, termed saliva-assisted transmission (SAT). The parasitic mite of honey bees, Varroa destructor, feeds on honey bee adults and pupae and is the vector for a variety of pathogenic organisms, such as Deformed wing virus (DWV) that has been attributed to global colony losses. Significant efforts have been put forth to define the ecological impacts of this virus as well as some behavioral changes to individual bees. However, the SAT induced by DWV infection and further, pro-feeding proteins in Varroa that are critical for successful feeding remains underexplored. Thus, we aimed to define secreted

proteins that are essential for mite feeding and the DWV-induced changes to secreted proteome. This was performed through PEAKS LFQ and LC-MS/MS to examine differences in protein abundance, identifying a total of 8,362 proteins from saliva which was cross-checked with the transcriptomic data of mite salivary glands. The secreted proteome of DWV-infected mites was significantly different when compared to uninfected mites with 17 being upregulated and 32 being downregulated. The upregulated proteins were distributed in higher expression ranks and mostly secretory proteins unlike the downregulated proteins, most of which are cell component proteins, suggesting the modulation is achieved by upregulation of proteins. The upregulated proteins include serine protease inhibitors, an orthologue of AV422, and salivary chitinase which are reported to be related to feeding behavior in ticks or the mite. Quantification of the feeding amount using fluorescent microscopy showed that knockdown of one unidentified secretory protein with the highest expression level via RNA interference appeared to reduce the feeding amount of the mites, which suggests these salivary proteins could be putative targets to prevent mite feeding or vector competence. Further analysis with the electrical penetration graph (EPG) will illuminate the role of the salivary proteins in facilitating feeding behavior and viral transmission.

44. CLIMATE VARIABILITY SHAPED THE COURSE OF THE BLACK DEATH AND SECOND PLAGUE PANDEMIC

Nicolas Gauthier - Florida Museum of Natural History, Departments of Geography and Anthropology, Emerging Pathogens Institute, University of Florida; **Aniruddh Chandrasekhar** - Florida Museum of Natural History, University of Florida; **Gabriela Hamerlinck** - Department of Geography, College of Liberal Arts and Sciences, University of Florida

Climate variability significantly shapes the spread and intensity of vector-borne diseases, influencing both vector competence and the population dynamics of potential hosts. However, the limited observational windows of contemporary climate data often fail to capture the multidecadal trends and oscillations that drive these dynamics. Here, we offer a long-term perspective on the complex interplay between environmental factors and disease transmission, examining how climate shaped the course of the Black Death and the centuries-long plague pandemic that followed. Using spatially explicit paleoclimate reconstructions, detailed historical records, and advanced computational models, we reveal recurring patterns of co-variability among climate, plague vectors, and their mammalian hosts in space and time. These findings provide an unparalleled view of how climate impacts vector-borne disease over the long term, offering valuable insights for future public health planning and preparedness strategies in the face of rapidly accelerating global climate change.

45. COMPARING ANTIGENIC MAPS OF DENGUE VIRUSES GENERATED FROM HUMAN AND AFRICAN GREEN MONKEY SERA

Gabrielle Johnson - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Kellie Goodnight** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Luke Trimmer-Smith** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Leah Katzelnick** - NIH National Institute of Allergy and Infectious Diseases, Bethesda, MD; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: Dengue fever is a vector-borne disease caused by the dengue virus belonging to the Flavivirus genus. It is endemic in over 100 countries, in regions where nearly half of the world's population resides. This leads to millions of reported cases, and around 40,000 deaths annually. From 2000 to 2019, the number of reported cases increased tenfold, making it the most prevalent as well as the fastest growing mosquito borne virus globally.

Antigenic cartography, a method for visualizing and examining immune response to pathogens, has been utilized in the study of dengue for several years. Past publications were able to find important trends in dengue by using sera from monotypic dengue infected African Green Monkeys (AGM), collected in highly controlled experiments. Due to the high cost and difficulty of replicating these animal trials to replenish this sera, it is important to find alternatives. Recent research has included sera collected from seropositive humans, but it has yet to be determined how well human sera compares to AGM sera for use in antigenic cartography.

Methods: Antigenic maps will be generated using neutralization titers. These were calculated from previously performed serological assays, Plaque Reduction Neutralization Tests (PRNT). PRNTs used different dengue viruses, neutralized by human or AGM sera, and were grown on C6/36 cells. Pairwise distances will be calculated for each set of viruses

for each map and these distances will then be compared using Pearson's correlation coefficient. Secondary assessments regarding how altering the amount of information used to generate maps influences outcomes will also be performed.

Results: An antigenic map created using 16 viruses and AGM sera will be compared to a map with the same 16 viruses and human sera with known infection histories. Additional tests will show how reducing the number of included viruses, including fewer sera samples, and/or limiting the number of viral variants can distort the map. Maps including humans with primary dengue infections will be compared to maps generated from secondary infection data.

46. COMPARISON OF HUMAN ANTI-AEDES SPP. MOSQUITO SALIVARY PROTEINS IGG IN THREE US POPULATIONS

Veronique Etienne - Department of Comparative, Diagnostic, and Population Medicine, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Berlin Londono-Renteria** - Department of Tropical Medicine and Infectious Disease, School of Public Health and Tropical Medicine, Tulane University; **Ruiyu Pu** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Estelle Martin** - Department of Entomology and Nematology, Department of Entomology and Nematology, Institute Of Food And Agricultural Sciences, University of Florida; **Liezl Farinas** - Department of Entomology and Nematology, Department of Entomology and Nematology, Institute Of Food And Agricultural Sciences, University of Florida; **Adriana Gallagher** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Rebecca Christofferson** - Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University; **Michael McCracken** - Viral Diseases Branch, Walter Reed Army Institute of Research; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Maureen Long** - Emerging Pathogens Institute, University of Florida

Introduction: Mosquito-borne diseases represent a significant global health challenge to human and animal health. Antibody responses to mosquito salivary proteins (MSP) offer a valuable tool for assessing population-level mosquito exposure. However, there has been limited exploration of cross-reactivity between responses to different mosquito species and within colonies of the same species. This study aims to assess variations in antibody responses to Ae. albopictus and Ae. aegypti across three geographically distinct regions with varying Aedes mosquito compositions.

Methods: The human populations included residents of North Florida, USA (Ae. albopictus); residents of South Florida, USA (co-circulating, overwhelmingly Ae. aegypti); and residents of Maine, USA (no known circulation of Ae. aegypti or Ae. albopictus, but presence of other Aedes spp.). Multiple in-house ELISAs were developed to measure human IgG responses to salivary gland extracts (SGE) from wild colonies of Ae. albopictus and Ae. aegypti, as well as a long-established lab colony of Ae. aegypti. Seasonality (high and low mosquito exposure) was also examined.

Results: South Florida exhibited significantly higher ODs during high and low mosquito prevalence compared to North Florida and Maine for all mosquito colonies ($p<.05$). South Florida had higher ODs during high and low mosquito prevalence as compared to Main and North Florida (Kruskal-Wallis with Bonferroni correction $p<0.0001$ for all), and there was only a significant difference between North Florida and Maine during the low mosquito prevalence period (Kruskal-Wallis with Bonferroni correction $p<0.05$), when using the difference between the two species' average OD scores. Additionally, a strong correlation between average anti-Ae. aegypti OD for the wild and lab strain was observed for Maine, North Florida, and South Florida across both mosquito prevalence levels ($R2 \approx 0.87$, $p<<<.05$ for all six combinations).

Discussion: The tropical climate in South Florida likely contributes to heightened mosquito bite exposure, reflected in the significantly higher average anti-MSP OD scores as compared to North Florida and Maine. Discernible differences in OD scores were evident primarily between

areas with high mosquito densities as compared to low/no mosquito presence. The robust correlation between IgG responses to SGE derived from wild and lab adapted mosquitoes suggests the suitability of using lab-adapted colonies for identifying key immunogenic proteins, potentially simplifying experiments by utilizing strains more amenable to artificial rearing. These findings underscore the suitability of using anti-SGE IgG for monitoring areas with high mosquito densities and emphasize its utility in tracking low-level mosquito invasions.

47. DETECTING THE IMPACT OF TEMPERATURE ON AEDES MOSQUITO TRAITS: A MODELING STUDY

Mondal Hasan Zahid - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Bernardo Garcia Carreras**; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: Temperature plays a significant role in the transmission of mosquito-borne diseases through its effects on mosquitoes' development, survival, reproduction, and biting rates. Earlier modeling works studied the thermal dependency of Aedes mosquitoes' life history trait parameters using experimental data to detect the impact of temperature on transmissions of dengue, Zika, and chikungunya.

Method: In this study, we develop a simple deterministic model to characterize the interplay between temperature and mosquito history traits using mosquito data collected in fields across counties in Florida during 2001-2013. In our analysis, we consider human density, in addition to temperature, as one of the drivers of mosquito abundance.

Results: Our estimation of these temperature-dependent parameters using field data shows replicating mosquito populations better than estimations using experimental data do. Thus, field data are found to be more useful for capturing the interrelation between temperature and life stages (eggs to adults) and biting rates of Aedes mosquitoes.

Conclusions: This characterization will help in studying the Aedes mosquito-human interaction and dengue transmission dynamics better. However, more modeling studies using different field datasets are suggested to establish the findings of this study.

48. EVALUATING NATURAL PRODUCTS AS REPELLENTS AND INSECTICIDE SYNERGISTS FOR AEDES AEGYPTI

Haley Dabbs - Department of Agriculture, Undergraduate Research, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Vilheim Piwowarek** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Bob Aldridge** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Jedidiah Kline** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Adam Bowman** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Seth Gibson** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Dan Kline** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Ken Linthicum** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture; **Edmund Norris** - Department of Agriculture, Emerging Pathogens Institute, College of Agricultural and Life Sciences, US Department of Agriculture,

Aedes aegypti mosquitoes are vectors of diseases such as yellow fever, dengue virus, chikungunya, and Zika. One way to stop the spread of these serious diseases is to eliminate or deter the mosquitoes that vector them. They can be controlled using insecticides/repellents such as pyrethroids, but resistance has been a growing concern. It is necessary to develop new formulations that can remain effective. Using natural products like essential oils in these formulations may be beneficial as the represent new chemistries that act in unique ways from pyrethroids currently on the market, and they are potentially safer to non-target

organisms. In this study, five natural oils were evaluated for their ability to repel and/or knockdown mosquitoes when used in conjunction with metofluthrin. Two oils increased metofluthrin repellency to 100% at 60 minutes, and 3 oils increased metofluthrin knockdown to over 90% in the same time frame. These data suggest these oils may be useful in repelling or immobilizing mosquito bites, and thus the spread of disease, without selecting for resistance in the way a traditional product would. In the field some of these oils were repellent when applied alone (without metofluthrin) indicating their potential as future pest control technologies.

49. EVALUATION OF SILVER NANOPARTICLES AS A CONTROL TOOL AGAINST ADULT MOSQUITO VECTORS

Kai Blore - Department of Entomology and Nematology, Anastasia Mosquito Control District; **Rebecca Baldwin** - Department of Entomology and Nematology, University of Florida; **Cameron Jack** - Department of Entomology and Nematology, University of Florida; **Christopher Batich** - Department of Materials Science and Engineering, University of Florida; **Whitney Qualls** - Anastasia Mosquito Control District; **Rui-De Xue** - Anastasia Mosquito Control District

Insecticides remain an integral component of mosquito control operations but sustained use of a limited number of active ingredients (AI) has led to widespread development of resistance. Development of novel insecticides, formulations and AIs will be necessary to maintain future efficacy of mosquito control. Towards this, toxicity screening of metal nanoparticles (AgNPs) was conducted via topical applications to assess their viability as potential adulticides against different genera of mosquitoes. Nanoparticles were synthesized from silver nitrate (AgNO3) using essential oils as both a reducing and capping agent to stabilize the AgNPs molecules. The resultant AgNPs were characterized by UV-Vis spectrophotometer analysis, transmission electron microscope and Zetasizer NSP to determine size and morphology. Different essential oil AgNPs were applied topically in conjunction with permethrin against adult mosquitoes of different genera to also evaluate potential synergism.

50. EXPLORING THE COMPOUND VULNERABILITY LANDSCAPE OF MOSQUITO-BORNE DISEASE IN FLORIDA

Gavriella Hecht - 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

Introduction: In Florida, mosquito-borne diseases (MBDs) pose a threat to public health, which is compounded by socioeconomic heterogeneity and impending shifts in risk due to climate change. In this study, 'vulnerability' describes the balance of ill-health burden, mediated by deprivation or access to interventions. To understand compound MBD vulnerability, and potential burden to vector management, an assessment of climate mediated MBD transmission suitability and community susceptibility was conducted. This study aimed to construct a Mosquito-borne Disease Vulnerability Index (MBDVI), integrating social vulnerability, thermal transmission suitability, and county vector control district (VCD) coverage, to explore geospatial MBD vulnerability in Florida. Using only publicly available data, an additional aim was to create a tool transferrable to other jurisdictions.

Method: This study combined a social vulnerability measure, thermal suitability of transmission (S(T), in months) for Zika and dengue, and VCD effort coverage, for Florida. Data included the CDC Social Vulnerability Index (SVI), S(T) model gridded outputs, and VCD geolocation data. The SVI evaluated community vulnerability at Census tract and county levels. MBD suitability data comprised published S(T) model gridded outputs as a function of climate. VCD data classified counties into three coverage categories, where coverage refers only to the ability to access coverage: full, partial, and none. Full coverage indicates all-county VCD access, partial indicates part-county access, and none indicates no access. Geospatial data layers were collated in R and ArcGIS Pro and rendered into a single index. Hotspot analyses were conducted using local Getis-Ord statistics to explore spatial heterogeneity for inputs and overall vulnerability.

Results: Social vulnerability was clustered in Florida, with high values in inland counties, the panhandle and in central Florida, indicated by significant hotspot clusters. MBD transmission suitability was also clustered with hotspots identified for Zika and dengue in south and central Florida. In 64 of 67 counties, at least one active VCD covered the entire county. Higher MBDVI values were concentrated in central and southern Florida, as well as some inland northern counties. Local Getis-Ord analysis identified significant VIMBD clusters encompassing 13 contiguous counties in southern Florida, extending to the southeastern-most coastline.

Conclusions: The MBDV index allowed geospatial identification of vulnerable hotspots in central and southern Florida. The combined index revealed clustering patterns which highlight a need for tailored interventions for MBDs within the state, and the role of different factors in vulnerability. This approach provides an initial step towards a more widely applicable tool for regional scale VCD planning.

51. HOW DO SOCIO-ECONOMIC FACTORS IMPACT MOSQUITO ABUNDANCE AND DIVERSITY IN SUBURBAN NEIGHBORHOODS?

Timothy D. McNamara - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Faith M Oi** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Peter Jiang** - Gainesville Mosquito Control Services; **John Diaz** - Department of Agricultural Education and Communication, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Clem Taylor** - Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Income has been shown to significantly impact the abundance of both Aedes and Culex mosquito populations, with lower-income communities experiencing higher mosquito abundance than high-income communities. Unfortunately, these low-income communities have also been shown to have higher rates of West Nile virus infection the mosquitoes present than higher-income communities. This difference in mosquito burden is

thought to be the result of a higher abundance in breeding sites in low-income areas due to the presence of more breeding sites. However, much of this research has focused on the impacts to urban systems, meaning that the impacts of poverty on mosquito burden in smaller communities is poorly understood. To bridge this gap, we are conducting a survey of mosquito abundance and diversity in 6 neighborhoods in Gainesville, FL of varying income. We are also conducting a Knowledge, Attitude and Practices (KAP) survey of the neighborhood residents to determine their perceived mosquito burden, knowledge of mosquito management practices, and to measure how education impacts practices to control mosquitoes in their area. Preliminary results indicate that Winter and early season (December to October) Aedes and Culex mosquito abundance did not vary by income.

52. IDENTIFICATION OF A NOVEL RHABDOVIRUS AND ORBIVIRUS IN A DEAD FARMED FLORIDA WHITE-TAILED DEER

Emily DeRuyter - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Disease, Emerging Pathogens Institute, University of Florida; **Kristen Wilson** - Department of Wildlife Ecology and Conservation, Emerging Pathogens Institute, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, 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

A novel ephemeral fever rhabdovirus and a new CHeRI orbivirus were identified in a dead farmed white-tailed deer (WTD; Odocoileus virginianus) in Florida. The virus genomes were sequenced using a NextSeq platform. Ephemeral fever, caused by ephemeral fever rhabdovirus, is an economically important disease which primarily affects cattle and water buffalo throughout tropical and semitropical environments of the Eastern Hemisphere. Morbidity associated with the

illness can be costly to farmers as it results in a decreased milk yield and reproductive losses. Noteworthy, there have been reports from some countries of outbreaks of ephemeral fever with mortality rates that exceed 20%. We previously discovered CHeRI orbiviruses 1 – 3 in dead farmed WTD, typically as a co-infecting agent with epizootic hemorrhagic disease virus. The CHeRI orbivirus in the animal of this report is a new variant that may be designated CHeRI orbivirus-4. Both CHeRI orbivirus and ephemeral fever virus are arthropod-borne viruses. This is the first known detection of ephemeral fever virus within North America and in WTD. Both of the viruses that were identified in this report may be transmitted by biting midges (Culicodes species), but other vectors such as mosquitoes and ticks are possible, and work must be performed to determine their natural vectors.

53. INTEGRATIVE MODELING OF PHYLODYNAMIC, VECTOR, HUMAN TRAVEL, AND EPIDEMIOLOGICAL DATA TO INFER ARBOVIRAL IMPORTATION AND LOCAL TRANSMISSION.

Massimiliano Tagliamonte - Interdisciplinary Center for Biotechnology Research, University of Florida; **Matt Hitchings** - Department of Biostatistics, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Melanie Cash** - 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; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Carla Mavian** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Introduction: Following the Zika virus (ZIKV) epidemic in the Americas, 1,089 travel-related ZIKV cases occurred in Florida between 2016-2017, together with 276 locally acquired cases in 2016. We used

epidemiological data collected in Florida during 2015-2017 to develop new models to infer temporality of importation and local transmission.

Method: We sequenced 25 ZIKV genomes from locally acquired and 38 travel-related cases and identified transmission clades with IQ-TREE adding 575 ZIKV publicly available genomes, including 49 from Florida. We inferred phylogeographic transmission in BEAST. Using epidemiological investigation as a gold standard, we determined the accuracy of models to classify cases as locally or travel-acquired. Posterior probabilities from phylogeographic analysis, sample collection time, vector abundance, travel volumes by cruise and air, and ZIKV prevalence were used as predictors in regression models.

Results: Five clades identified multi-country independent introductions of ZIKV in Florida. Highest likelihood of importation and local transmission were estimated to be between January and April from Brazil, April to May from Colombia, April to August from Dominican Republic and Honduras, in April and July from Suriname, Guadalupe, Guatemala, Jamaica, Mexico, and Nicaragua, and in October from Puerto Rico. Regression models predicted importation status with an accuracy of 93% using phylogeography-inferred origin country and time of sequence sampling.

Conclusions: Multiple ZIKV introductions occurred at several points in time that led to local transmission foci in urban Florida settings. Time to the most common ancestor of locally acquired cases was months before the first reported local transmission suggesting undetected transmission and highlighting the need for prediction models that allow early detection and interventions. Our likelihood model infers countries as source of ZIKV importations in Florida and the temporality for local transmission in Florida. We plan to expand the current models to predict effect of change in climatic condition on risk of arboviral importation and local transmission.

54. MAPPING GEOGRAPHIC AND DEMOGRAPHIC SHIFTS FOR CONTAINER BREEDING MOSQUITO-BORNE DISEASE TRANSMISSION SUITABILITY IN CENTRAL AND SOUTH AMERICA IN A WARMING WORLD

Sadie Ryan - Department of Geography, Florida Climate Institute, College of Liberal Arts and Sciences, University of Florida; **Catherine A. Lippi** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Anna M Stewart-Ibarra** - Inter-American Institute for Global Change Research (IAI), Montevideo, Uruguay

The recent Intergovernmental Panel on Climate Change Sixth Assessment Report (IPCC-AR6) brought into sharp relief the potential health impacts of a changing climate across large geographic regions. It also highlighted the gaps in available evidence to support detailed quantitative assessments of health impacts for many regions. In an increasingly urbanizing world, there is a need for additional information about the risk of mosquito-borne diseases from vectors adapted to human water storage behavior. Specifically, a better understanding of the geographic distribution of disease risk under different climate warming scenarios and human population shifts. We present novel geospatial descriptions of risk for transmission for five mosquito-borne disease systems under future projected climate and demographic scenarios, including the potential risk for malaria in the event of the introduction and establishment of a vector of high global concern, Anopheles stephensi. We then present country-level and IPCC geospatial sub-regional risk descriptions under baseline and future projected scenarios. By including demographic projections using the shared socioeconomic pathway (SSP) scenarios, we capture potential future risk in a way that is transparent and straightforward to compare and replicate. The goal of this paper is to report on these model output data and their availability. From a sub-regional perspective, the largest proportional gains in risk will be seen in the Southwestern South America (SWS) sub-region, comprising much of the southwestern coastline, for which the suitability for Aedes aegypti-transmitted dengue and Zika will see massive increases with warming, putting a large number of people at risk under future scenarios. In contrast, at the country level,

the largest projected population impacts will be seen in Brazil for both arboviral and potential introduced malaria risk, despite some risks projected to decrease as parts of the country are too hot to sustain transmission. This paper provides modeled outputs for future use, in addition to broad summary descriptions at regional and country levels.

55. MAPPING POTENTIAL TRANSMISSION RISK OF EASTERN EQUINE ENCEPHALITIS (EEE) VIRUS IN FLORIDA IN A CHANGING ENVIRONMENT

Emily Peterson - University of Florida; **Catherine A. Lippi** - 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

Climate change has the ability to exacerbate the spread of disease, especially mosquito-borne diseases, as mosquito behavior and survival are heavily impacted by ambient temperature conditions. This includes Eastern equine encephalitis (EEE) virus, a pathogen primarily transmitted by Culiseta melanura mosquitoes to birds. Cu. melanura and other mosquito bridge vector species can drive spillover events in humans and equids, which are dead-end hosts. This is problematic, as EEE causes disease with very high mortality rates in both horses and humans. While there is not currently a pharmaceutical intervention available for humans, there is a vaccination that is effective in horses. This is a particularly salient veterinary public health issue in Florida, where a high concentration of horse farms occurs across the central portions of the state, particularly in Marion county, which is home to a thriving and nationally recognized horse industry. In this study, we aimed to predict thermal transmission suitability for EEE in north-central Florida under baseline conditions and future climate change scenarios. Using thermal transmission suitability data from existing literature with current and projected future climate data fromWorldClim, we mapped the number of months with temperatures suitable for EEE transmission, under current conditions and for the year 2030. We overlaid this on geolocated horse farms to explore potential risk to the concentration of Florida's horse industry. Areas with suitable temperatures for EEE transmission are

predicted to increase throughout the region, leading to prolonged periods of seasonal transmission risk.. This information can be used to inform future veterinary efforts to vaccinate horses against EEE and mosquito intervention and control.

56. MAPPING THE ANTIGENIC RELATIONSHIPS BETWEEN ZIKA & DENGUE

Luke Trimmer-Smith - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Leah Katzelnick** - NIH National Institute of Allergy and Infectious Diseases, Bethesda, MD; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Dengue and Zika exhibit numerous common characteristics. Both belong to the Flaviviridae family, often produce indistinguishable disease symptoms in humans, have overlapping geographic distributions, and share vector species. These also include antigenic similarities, where in some cases the antigens of one virus can produce neutralizing antibodies in individuals infected with the other virus (cross-reactivity). These overlaps can have real and potentially confounding implications on virus evolution, disease outcomes, treatment strategies, and vaccine efficacy. We utilize a panel of 20 single dengue-virus-infected African Green Monkey (Chlorocebus sabaeus) antisera and C6/36 mosquito cells (Aedes albopictus) to conduct serological assays, Plaque Reduction Neutralization Tests (PRNT), to assess the antigenic relationship between each sera/virus pair using virus variants (strains) from all four dengue serotypes. Over 400 outcomes have been generated from sequenced and genotyped dengue virus isolates collected over multiple decades, 1980s-2010s. Using antigenic cartography, the antigenic diversity based on the PRNT outcomes is used to construct a multi-dimensional map. Each stain is represented as a point on a map with the distance between each point corresponding with the antigenic uniformity between each strain. The more antigenically similar viruses group together in three-dimensional space with the members of each of the four dengue serotypes forming four distinct clusters with the more antigenically distinct members of

each serotype falling on the periphery of their respective cluster. To examine if and where Zika falls on the map, we conducted PRNTs using the same Green Monkey sera and four Zika isolates collected during the 2015-2016 outbreak in the Americas. Somewhat surprisingly, neutralization occurred in the PRNTs for each Zika variant, with neutralization not limited to a single dengue serotype or subtype but each Zika eliciting some detectable neutralization across most of the panel of sera. By employing antigenic cartography, the location of Zika points formed a group on the dengue map in close proximity to the DENV-4 cluster. We hope to continue to expand the map by conducting further PRNTs using additional arboviruses and by increasing the panel of sera to include more non-human primates and humans with well-described arbovirus infection histories.

57. MODELING HOST-PARASITE INTERACTIONS IN MALARIA BLOOD-STAGE INFECTIONS IN RHESUS MACAQUES

Luís L. Fonseca - Department of Medicine, College of Medicine, University of Florida; **Chester J. Joyner** - Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, USA; **Celia Saney** - Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, USA; **Alberto Moreno** - Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, USA; **John W. Barnwell** - Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, USA; **Mary R. Galinski** - Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, USA; **Eberhard O. Voit** - The Wallace H. Coulter Department of Biomedical Engineering at Georgia Institute of Technology and Emory University, Atlanta, USA

Introduction: Malaria is globally the most deadly parasitic disease in humans, and the long-time coexistence with malaria has left indelible marks in the human genome that are the causes of a variety of genetic disorders. Anemia is arguably the most common and severe complication of malaria, yet the root causes and mechanisms involved in the pathogenesis of malarial anemia are unclear and very difficult to study in humans. Non-human primate (NHP) model systems enable the

mechanistic study and quantification of underlying, causative factors of malarial anemia, and particularly the onset of severe anemia.

Materials and Methods: A discrete recursive model was developed to simulate host-parasite interactions during the blood stage infection; it accounts for reticulocytes, red blood cells (RBCs), and infected RBCs. The parameters of this mechanistic model were optimized against the readouts of individual macaque data, which had been obtained in the course of Plasmodium coatneyi and P. cynomolgi infections of cohorts of malaria-naïve rhesus macaques (Macaca mulatta). The model allowed detailed estimations of the levels of erythropoietic output, reticulocyte lifespan, RBC removal, and the immune response against the parasite in each macaque.

Results and Discussion: The results showed that rhesus macaques have a response to a P. cynomolgi infection that is difficult to understand: As expected, the infection resulted in anemia, yet 60% of the RBCs were lost by a mechanism other than parasite invasion, which is known as bystander effect. To compensate for the severe anemia, the host released younger reticulocytes and increased the erythropoietic output. These responses, however, appeared to be poorly coordinated, as the release of younger reticulocytes occurred too early, namely, while anemia had not yet set in, thereby probably aiding the parasite more than the host. Additionally, increased production of RBCs was only detected after treatment that lowered the parasitemia. The model also showed that, similarly to humans, reticulocytes in rhesus macaques circulate for about 24h before becoming mature RBCs.

Conclusions: Anemia, as a sequela of malaria, was due in 60% to bystander destruction of RBCs, and by an inability of the host to up-regulate erythropoiesis before suppression of parasitemia.

58. MULE-DEER POXVIRUS TRANSMISSION AND DETECTION

Lillian Maxwell - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, 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; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Kuttichantran Subramaniam** - Department of Pathology, Immunology, and Laboratory Medicine, College of Veterinary Medicine, University of Florida

Mule-Deer Poxvirus (MDPV) is one of the most common viruses on Florida White-tailed deer farms. Like many other prevalent deer viruses, this emerging pathogen causes high mortality among fawn populations. While recently characterized, little about the disease including transmission is unknown; however, preliminary data shows that MDPV outbreaks occur seasonally, similar to patterns of vector-borne disease. Other poxviruses typically transmit mechanically through vectors. With this information, we collected 89 Muscoid and Tabanus flies from two Florida deer farms and tested them for MDPV DNA to understand preliminary vector competency. In addition to vector transmission, we aimed to understand the resiliency of MDPV in the environment and whether transmission is possible through environmental reservoirs. Water and soil samples were collected, and environmental DNA (eDNA) was extracted in order to determine viral load in the environment. These neoteric methods will allow us to diversify our detection of viral pathogens using passive techniques. With this broader understanding of the environment's role in disease transmission, we will not only be able to identify disease presence faster but in a minimally invasive way. A greater understanding of poxvirus transmission is extremely important to protect the health of farmed animals. In the end, we want this information to be used by farmers and population managers to benefit not only heard health but the health of wild populations as well.

59. SEROLOGICAL AND GENETIC CHARACTERIZATION OF DENGUE TRANSMISSION DYNAMICS IN NIGER

Assao neino Bachir - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Matt Hitchings** - Department of Public Health, Emerging Pathogens Institute, College of Medicine, University of Florida; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Dengue poses a significant public health challenge in Africa, as evidenced by the 200,000 suspected cases and 782 reported deaths across 15 countries in 2023. However, this burden is likely underestimated due to limited surveillance and reporting capabilities in many nations.

Various factors influence Dengue transmission in Africa, including climatic conditions, vector ecology, human mobility, urbanization, socio-economic status, and co-circulation of other arboviruses. These factors exhibit temporal and spatial variations, resulting in heterogeneous and dynamic risk patterns.

Effective Dengue prevention and control in Africa necessitate a comprehensive, integrated approach, considering spatial and temporal dimensions, as well as interactions between the virus, vector, host, and environment. Spatial models, serological studies, genetic analysis, and mathematical models are instrumental in characterizing and predicting Dengue transmission dynamics.

Despite the report of the first introduction of DENV-3 in 2022 and the identification of a DENV-1 infection in a French traveler who visited Niger in 2016, Niger, a West African country, lacks formal Dengue studies, revealing gaps in data availability, quality and comparability. A comprehensive understanding of arbovirus transmission dynamics is critical, highlighting the need for systematic, collaborative studies to inform policies, practices, and intervention impacts.

We propose a serology study aimed at comprehensively understanding Dengue transmission in Niger. This includes investigating serotype-

specific circulation, age-specific seroprevalence (in urban and rural settings), historical force of infection, and the impact of migration. Additionally, genetic sequencing of DENV in Niger is proposed to confirm distribution, origin, diversity, and evolution, contributing to global Dengue epidemiology, genetics, and the development of effective vaccines and therapeutics.

This study will employ cross-sectional serological surveys across diverse regions in Niger to investigate Dengue transmission dynamics. Plaque Reduction Neutralization Tests (PRNT) will discern serotype-specific circulation. Urban and rural stratified random sampling will guide seroprevalence surveys, with catalytic models estimating historical force of infection. Incorporating travel history and mobility patterns will elucidate migration's impact on Dengue spread. Genome sequencing and phylogenetic analysis will be conducted to characterize the molecular epidemiology, evolution, and diversity of DENV in Niger.

It's important to note that this study has not commenced yet and is in the planning phase.

60. SINGLE CELL TRANSCRIPTOMICS AS A TOOL TO IDENTIFY NOVEL SELECTIVE ANTIFEEDANT TARGETS IN HEMIPTERAN PESTS

Flinn M. O'Hara - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Bryony C. Bonning** - Department of Entomology and Nematology, College of Agricultural and Life Sciences, University of Florida; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Species such as the southern green stink bug, Nezara viridula, are particularly problematic in agricultural landscapes due to their polyphagous behavior and resistance to classical chemical insecticides. High levels of insecticide resistance to most commercialized insecticides have stimulated efforts to identify novel approaches to control pest populations. Feeding is a tenant of survival for all animals and thus, it is surprising no commercialized insecticide specifically targets feeding

biology of arthropod pests. Stink bugs feed by inserting their piercing-sucking mouthparts into plant tissues through physically rupturing cells or secreting 'gel' saliva that forms a salivary sheath around the stylets to feed on phloem bundles. In addition, 'watery' saliva contains digestive proteins and is secreted to digest plant cell contents prior to ingestion. Therefore, the stink bug salivary gland and secreted saliva are critical to feeding events and plant digestion that indicates the development of products that inhibit function of secreted proteins or reduce the secretory activity of the stink bug salivary gland are likely to inhibit feeding. However, an incomplete understanding regarding the cellular composition and druggable targets of stink bug salivary glands remain. The development of synthetic chemicals targeting agricultural pests has been met with regulatory challenges resulting from potential negative impacts to beneficial insects, particularly the honey-bee, that hinder commercialization of novel mechanism insecticides. Therefore, the aim of this study is to define the expression profile of individual cells of accessory and principal salivary glands known to be relevant for protein secretion. It could then be possible to identify putative targets not expressed in the honey-bee genome that effect the secretory activity of the stink bug salivary gland and disrupt feeding. Whole salivary glands were dissected from a total of 10-15 stink bugs and pooled for each replicate. Whole tissues were then dissociated and single-cell sequencing of N. viridula salivary glands were performed for three replicates. Preliminary analysis of the raw single cell data has led to the identification of 15-18 cell clusters, each potentially representing a different cell type. Further dissection of these clusters has identified a minimum of 3 secretory cell clusters each containing a unique mixture of secreted. Furthermore, each cluster contains multiple potential targets that we intend to compare to the honey-bee genome. It will be possible to build on these initial findings through the design of in situ hybridization experiments to correlate clusters with cell types in whole accessory and principal glands.

61. SPATIOTEMPORAL PATTERNS OF THREE MOSQUITO SPECIES IN HAITI, 2018-2019

Ian Pshea-Smith - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Bernard Okech** - Uniformed Services University; **Ian Sutherland** - Navy Entomology Center of Excellence; **James Dunford** - Navy Entomology Center of Excellence; **John So** - Navy Entomology Center of Excellence; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Michael E. fr** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: We investigated the distribution and spatiotemporal clustering of Aedes aegypti, Ae. albopictus and Culex quinquefasciatus mosquitoes in three communal sections along Haiti's coast, focusing on their roles as vectors for viruses causing dengue fever and West Nile fever. All three of these mosquito species are present in Haiti and determining their spatiotemporal distributions is crucial for tailoring vector-borne disease intervention strategies.

Methods: The mosquitoes were caught using CDC Gravid, CDC Light, and BG Sentinel traps from August 2018 to September 2019. In total, 337 successful collection events were analyzed from 12 trap sites. Kernel density estimation (KDE) for mapping mosquito concentrations and space-time permutation models in SaTScan were employed for data analysis.

Results: KDE identified a primary hotspot (high concentration of counts) for each mosquito species at the intersection of the three communes. The space-time permutation models revealed consistent spatiotemporal clusters for all three species, with a cluster during January 2019 as the primary cluster for both Ae. aegypti and Cx. quinquefasciatus; this cluster encompassed four trap sites for Ae. aegypti and a single site for Cx. quinquefasciatus. For Ae. albopictus, the primary cluster occurred in November 2018, encompassing three trap sites in the northwest of the

study area. Both the January 2019 and November 2018 clusters were significant but not primary for the other mosquito species. Additional significant clusters occurred during May 2019 for one centralized trap site across all three outcomes. For Ae. albopictus, there was an additional cluster during August of 2018 across four trap sites in the south for Ae. albopictus. Cx. quinquefasciatus had a cluster that occurred at one trap site in the south from August 2018 through January of 2019.

Conclusions: We found some differences in the distribution and spatiotemporal patterns of Aedes aegypti, Ae. albopictus and Culex quinquefasciatus in a small periurban area of Haiti. These variations could be attributed to factors such as feeding preferences and environmental conditions, of which prominent features such as altitude may play a significant role in this setting, although this remains to be elucidated. This research may underscore the importance of identifying mosquito hotspots for targeted disease control interventions, as different mosquito species exhibited different spatiotemporal patterns. These analyses provide critical insights for public health policy and vector management strategies in Haiti.

62. STRUCTURE-ACTIVITY RELATIONSHIP STUDIES REVEAL POTENT ANTIBIOTIC ACTIVITY OF TOLFENPYRAD DERIVATIVES THAT TARGET FRANCISELLA

Nimra Khalid - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Nicole Adams** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Aya Taki** - University of Melbourne, Parkville, Victoria, Australia; **Robin Gasser** - University of Melbourne, Parkville, Victoria, Australia; **Jonathan Baell** - Monash University, Parkville, Victoria, Australia; **Aria Eshraghi** - Department of Infectious Disease, College of Veterinary Medicine

Francisella tularensis is a highly infectious and deadly intracellular bacterial pathogen that establishes an infection with few as ten viable bacteria. Due to its extreme virulence and potential for weaponization, the identification of new antibiotics against Francisella is essential for global health security. We previously discovered that Tolfenpyrad, a pesticide used worldwide, displays antimicrobial activity that specifically targets Francisella species. To improve safety and potency, we performed structure-activity relationship studies on 262 synthesized analogs based on the Tolfenpyrad scaffold. Though these analogs have diverse chemical structures, none of them exhibits activity toward closely related Gram-negative bacteria. Mutations in two genes that make Francisella resistant to tolfenpyrad also confer resistance to these compounds, indicating that they share a common mechanism of action with Tolfenpyrad. Compared to Tolfenpyrad, 10 compounds display greater than 5-fold better antibiotic activity. We employed stringent conditions to evaluate the toxicity of these compounds to cultured macrophages and found three drugs that demonstrate potent efficacy against Francisella without affecting viability of host cells. These compounds potently inhibit intracellular growth of Francisella in immortalized bone marrow-derived macrophages. These compounds not only serve as promising candidates for the development of anti-Francisella therapeutics but also provide valuable tools for uncovering the intricate mechanisms of bacterial pathogenesis. Future discovery of the bacterial target(s) of these drugs

will open doors to the development of targeted interventions against this highly virulent pathogen.

63. SUSCEPTIBILITY OF AEDES SPECIES TO FORMULATED INSECTICIDES IN ST. JOHN'S COUNTY, FLORIDA

Connor Kuppe - Anastasia Mosquito Control District; **Whitney Qualls** - Anastasia Mosquito Control District; **Olivia Sypes** - Anastasia Mosquito Control District; **Vindhya Aryaprema** - Anastasia Mosquito Control District

Two container inhabiting species, Aedes aegypti and Aedes albopictus, competent vectors of yellow fever, dengue, and chikungunya, are a persistent species of concern within St. Johns County (SJC), Florida. Within SJC, Anastasia Mosquito Control District (AMCD) is charged with reducing the local risk of mosquito-borne diseases. As an Integrated Mosquito Management Program, control is conducted through various methods, however, insecticide application remains the most efficient. As such, susceptibility of local mosquito populations within the county, to formulated insecticides, remains an active concern. For this reason, a comprehensive investigation of insecticide resistance status of different local populations of Ae. aegypti and Ae. albopictus was established. Populations from five pre-designated operational zones (C01, C02, C06, C10, and Evergreen Cemetery) were chosen based on population abundance and treatment pressure. Eggs for Ae. aegypti and Ae. albopictus were collected in 2022 and 2023 seasons and reared to F1 and F2 adults. Formulated products, Duet® (Prallethrin 1% + Sumithrin 5% + Piperonyl Butoxide 5%) and Aqualuer 20-20® (Permethrin 20.6% + Piperonyl Butoxide 20.6%), were tested by topical application bioassays to determine LD50. Parallel assays were conducted on susceptible colony reared Ae. aegypti (Orlando 1952 Strain) and Ae. albopictus (Gainesville 2003 Strain) to determine resistance ratios (RR). Treatment zones which displayed high RR were additionally tested through CDC bottle bioassays in order to establish a comparison and confirmation of topical application bioassay results. Topical application bioassays conducted on Ae. albopictus from zone C01, against Aqualuer, resulted in a RR of 41.42, and the resistance status was confirmed through a bottle bioassay. Ae. aegypti from zone C01 and C02, against Duet, resulted in a RR of 102.75

and 65.37, respectively. Ae. aegypti from C02 also displayed resistance against Aqualuer, with a RR of 80. Due to sampling limitations, no CDC bottle bioassay confirmations were secured on Ae. aegypti from these zones. All other populations tested displayed low to no resistance (RR<2.5). This study illustrates how insecticide resistance can form differentially in subpopulations within the same geographical area and demonstrates the importance of establishing a long-term insecticide resistance monitoring plan.

64. THE FLORIDA KISSING BUG (HEMIPTERA,TRIATOMINAE) – UNEARTHING A POTENTIAL NEW SPECIES OF THE CHAGAS VECTOR

Norman L. Beatty - Department of Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Sebastiàn Pita** - Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay; **Bruno A. S. Ruidíaz** - Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay; **Francisco Panzera** - Sección Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay; **Keswick Killets** - Department of Veterinary Integrative Biomedical Sciences, Texas A&M University College Station, Texas, USA; **Rachel Curtis** - Department of Veterinary Integrative Biomedical Sciences, Texas A&M University College Station, Texas, USA; **Gabriel L. Hamer** - Department of Entomology, Texas A&M University, College Station, Texas, USA; **Sarah A. Hamer** - Department of Veterinary Integrative Biomedical Sciences, Texas A&M University College Station, Texas, USA

Introduction: Kissing bugs (triatomines) are the blood-sucking vectors of the parasite, Trypanosoma cruzi, which is the causative agent of Chagas disease. Triatoma sanguisuga (Leconte) is a kissing bug found throughout the eastern United States (US) and described in Florida. Historical records (Mead, 1965) document the Florida T. sanguisuga as being morphologically distinct and was previously considered its own subspecies, Triatoma sanguisuga ambigua (Neiva). The most current and widely accepted Triatominae entomological key (Lent & Wygodzinsky, 1979) discusses these distinct findings in Florida, but authors suggested

that further study is needed before recognition of the subspecies or even being considered its own Triatoma species.

Methods: Genomic DNA was extracted from the legs of Triatoma sanguisuga specimens collected from 29 distinct counties in Texas and 8 counties in Florida. The mitochondrial protein-coding gene cytochrome b (cytp) was amplified using primers CYTB7432F and CYTB7433R. PCR was carried on using Taq® 2x master mix kit. PCR products were Sanger-sequenced by Macrogen Inc. MAFFT v7.453 was employed to align DNA sequences. Genetic distances were calculated using APE package v5.4.1 in R v3.6.1, using the Kimura 2-parameter substitution model (K2-p). Maximum likelihood (ML) phylogenetic tree was obtained using PhyML v3. Bootstrap values were estimated from 1000 pseudoreplicates. Using the dataset from this collection and other known sequences in NCBI Genbank, we then used the Generalized Mixed Yule Coalescent (GMYC), hierarchical approach using Bayesian model-based DNA sequence clustering (hierBAPS), and Maximum Likelihood (ML) phylogenetic tree methods to evaluate species delimitation within Triatoma sanguisuga.

Results: ML tree, GMYC and hierBAPS clustered data analysis reveal 4 unique clades of Triatoma sanguisuga. Florida isolates were found only in our state and distinct from the other clades with an overall genetic difference of 8.36%. Two clades were found in Texas. One Texas clade being restricted to the middle and southeast regions of the state and the other was widely dispersed in other portions of the eastern US along with the third clade.

Discussion: These preliminary results support genetic diversity within Triatoma sanguisuga, revealing at least 4 clades. The Florida clade carries enough genetic diversity to be considered its own species, but more research is needed. This could include whole genome sequencing and morphological mapping of the known 4 clades, as well as breeding experiments to assess whether these clades are reproductively compatible.

65. THE GEOGRAPHIC DISTRIBUTION AND ENVIRONMENTAL DRIVERS OF ORNITHODOROS TURICATA THROUGH THE SOUTHEASTERN UNITED STATES

Sebastian Botero-Cañola - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Carson Torhorst** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Nicholas Canino** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kathleen O'Hara** - Center for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, USDA; **Angela James** - Center for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, USDA; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

The soft tick Ornithodoros turicata (O.t.) is a component vector for African Swine Fever (ASF), a globally spreading viral disease affecting wild and domestic swine. Despite its epidemiological importance, there are large gaps in our understanding of the distribution and ecology of O.t. This is especially true for Florida, where only a handful of published occurrence records of the species exists, yet the risk of ASF introduction and establishment is high. In this work, we integrated historical occurrence data with systematic surveys to i) predict the distribution of O.t. over the southeastern USA; and ii) Assess the influence of environmental conditions over habitat suitability for this species. Our sampling targets to evaluate the occurrence of O.t were the burrows of gopher tortoises, the preferred microhabitat of this tick in the region, and which we surveyed by vacuuming a soil sample from its interior. We Employed data on the detection probability of O.t to determine the number of sites to survey and the sampling intensity per site required to optimally cover Florida’s environmental gradients while maximizing the odds of detecting the tick. This resulted in a sampling scheme comprising

the survey of five burrows at 100 sites. This effort allowed the detection of O.t. at 58 sites, notably increasing the occurrence records for the eastern population of the species. Using these records, in conjunction with GIS layers describing climatic conditions and soil composition, we created a Maxent model to predict habitat suitability for the species through the Southeastern USA. The model displayed a good performance at predicting the species distribution (ROC-AUC = 0.96) and suggests that 85% of Florida presents suitable conditions for the species. O.t is predicted to occur north of Lake Okechoobee and east of the western extreme of the panhandle. In addition, southeastern Georgia presents a block of suitable habitat. Through a Jackknife analysis, we found that soil composition in addition to precipitation seasonality and temperature are key variables in shaping the distribution of the species. This work highlights the importance of tick surveillance in the event of ASF introduction as the broad distribution and prevalence of O.t. over the landscape, in addition to large populations of feral swine, may enhance the risk of the sylvatic establishment of this pathogen.

66. TICK BITE: EVALUATING EXPOSURE TO TICKS BY MEASURING ANTIBODY IN HUMANS TARGETING TICK SALIVA IN A PROSPECTIVE COHORT

Adriana Gallagher - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **J. Glenn Morris, Jr.** - Emerging Pathogens Institute, University of Florida; **Maureen Long** - Emerging Pathogens Institute, University of Florida; **Jesus Valenzuela** - National Institute of Allergy and Infectious Diseases; **Lucas Tirloni** - National Institute of Allergy and Infectious Diseases; **Albert Ko** - Yale University; **Anna Durbin** - John Hopkins University; **Adam Waickman** - SUNY Upstate; **Stephen Thomas** - SUNY Upstate; **Derek Cummings** - Department of Biology, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Lyme disease is a big concern regarding tick borne diseases, espcially in the Northeastern United States. It is carried by Ixodes scapularis infected with Borrelia burgdorferi. People typically get infected while walking outside in tall grass, where ticks can attach to their skin. The ticks spread

this disease through their saliva and which starts an antibody reaction. Estimating the risk of infection is difficult but it also important to quantify individual-level risk exposure to I. scapularis and the risk factors associated with tick bites across populations and geographic areas. Our study is designed to understand the risk everyday people have to exposure to tick bites. The study is divided into two objectives, the first being assay development and the second an epidemiological assessment. We will use a novel enzyme-linked immunosorbent assay (ELISA) to evaluate antibody reactions to tick bites. The ELISA will use I. scapularis saliva as coating antigen. For the assay development we will use samples collected retrospectively from different geographic areas in the United States. These areas have been selected for their high or low incidence of tick bites. The total number of samples tested for the first objective is 3500, which will let us establish optical density (OD) cut-offs and find a negative control. Negatives will be established using samples from Colorado, Texas, and Florida which are areas free or mostly free of I. Scapularis. Seronegativity will be confirmed with Western Blot and then it will be used as a negative control for the rest of the study. Seropositive samples will come from areas of high incidence of I. Scapularis, Connecticut, New York, and Maryland. For the second objective, we will enroll 200 participants in 4 sites. The sites will consist of 3 high incidence areas, Connecticut, New York, and Maryland, and one low incidence area being Florida. Participants will be asked to come in twice to donate blood and complete a survey , once in the pre-exposure season (March-May) and another in the post-exposure (August-November). All participants' samples will be run on the novel ELISA to measure their antibodies. This information along with the survey responses from participants will provide a better understanding of behaviors and geographic areas that have a greater risk of exposure to ticks and Lyme Disease.

67. TOWARDS UNDERSTANDING RICKETTSIA PARKERI SURVIVAL AND RESPONSE TO OSMOTIC FLUX

Louise M. Ball - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Rickettsia parkeri is a Gram negative, zoonotic obligate intracellular bacterial pathogen responsible for maculatum disease in humans. R. parkeri is maintained in the Gulf Coast tick (Amblyomma maculatum) and transmitted to humans during blood feeding. Within the midgut of the tick vector, R. parkeri is exposed to osmolarity ranges between ~300 and ~600 mOsmoles during the stages of feeding. In infected humans, R. parkeri initially infects macrophages and dendritic cells. After distribution to the lymph nodes, the bacteria spread throughout the host via blood and infect endothelial cells in several organs and skin. Therefore, while in the human host, R. parkeri experiences a more stable osmolarity of ~300 mOsmoles.

In this project we will investigate the impact of osmotic flux on R. parkeri growth and survival in human and tick cell tissue culture. Preliminary results have shown that bacterial plaque size is reduced in human endothelial cells when incubated in ~200 mOsm medium. Quantitative PCR on R. parkeri mRNA from infected human endothelial cells showed upregulation of R. parkeri genes MC1_04185, MC1_01270 and sigma factor RpoD after growth in ~200 mOsm medium. R. parkeri like most obligate intracellular bacteria, experienced “genome reduction” and is dependent on the host cell for many metabolic pathways. The genome contains several hypothetical or pseudogenes, and non-coding DNA. MC1_04185 is annotated as an amino acid transporter. Bioinformatics reveal that the MC1_04185 gene product exhibits 24.2% identity and 42.7% similarity with a potassium transporter (KimA) found in Bacillus subtilis. The predicted tertiary structure aligns with the KimA crystal structure and key residues required in KimA for function are conserved in MC1_04185. As genetic manipulation is difficult in R. parkeri, we will

attempt to utilize E. coli mutants deficient in potassium uptake as a surrogate system to observe potassium transport capability by MC1_04185.

To fully understand the differences between R. parkeri overall gene expression in response to osmotic flux, we will map the transcriptome of R. parkeri grown in hypertonic (~800 mOsm) and hypotonic (~200 mOsm) medium in Amblyomma tissue culture cells (AAE12) and human endothelial cells (EA.hy926). Of special interest are modulation of virulence factors, metal transporters and secretion system genes. Studies have showed that in another tickborne pathogen, Borrelia burgdorferi, many of these genes are upregulated in low osmolarity medium.

Future directions include transcriptomics on live tick vectors, comparing bacterial and arthropod transcripts between ticks infected with R. parkeri or the endosymbiont Candidatus R. andeanae.

68. UTILIZING THE DIHYDROFOLATE DESTABILIZATION DOMAIN (DDD) SYSTEM FOR CONDITIONAL PROTEIN EXPRESSION IN AEDES AEGYPTI AND ANOPHELES STEPHENSI: A NOVEL APPROACH TO ENHANCE GENETIC CONTROL STRATEGIES

Victoria Mills - Department of Entomology and Nematology, Florida Medical entomology Laboratory; **Ivana Saborit** - Department of Entomology and Nematology, Florida Medical Entomology Laboratory; **Mathieu Zamy** - Department of Entomology and Nematology, Florida Medical Entomology Laboratory; **Bianca C. Burini** - Department of Entomology and Nematology, Florida Medical Entomology Laboratory

The escalating threat of mosquito-borne diseases necessitates innovative genetic control strategies for vectors such as Aedes aegypti and Anopheles stephensi. This study focuses on establishing a protein expression conditional regulatory system using the dihydrofolate destabilization domain (DDD) system based on a mutated E. coli dihydrofolate reductase (DHFR) destabilization domain. The DDD system, stabilized by the folate analog trimethoprim (TMP), operates at the protein level, enabling rapid and reversible protein knockdown, making it more advantageous than traditional DNA- or RNA-based strategies.

The DDD system involves fusing the protein of interest (POI) to a tag, directing it to degradation pathways; however, TMP prevents degradation, offering precise control over protein expression. This holds relevance in genetic strategies for population suppression, especially in scenarios where POI needs to be expressed in mosquitoes for mass release programs but must be absent during the rearing process in biofactories. To evaluate the system's efficacy, we assessed its impact on the life history traits of mosquitoes, including fecundity, fertility, survival, and pupation time.

In initial experiments with Aedes aegypti, larvae reared in three TMP concentrations (0.1mM, 1mM, and 10mM) demonstrated a concentration-dependent delay in pupation time, suggesting the DDD system's influence on mosquito development. Subsequent transgenic engineering of mosquitoes involved linking the DD domain to a green fluorescent protein (GFP) and assessing its degradation in the absence of TMP will determine if this system is suitable for mosquitoes.

This research underscores the transformative potential of the DDD system as an influential tool for achieving conditional protein expression in mosquito vectors. The versatility of this system, which can be employed to either stabilize detrimental proteins in transgenic mosquitoes as required or facilitate their targeted degradation, positions it as a promising candidate for widespread implementation in genetic control strategies. The adaptability and precision offered by the DDD system mark a significant advancement, paving the way for environmentally responsible interventions to mitigate the impact of mosquito-borne diseases on a large scale.

69. VIRUS-INDUCIBLE DEATH TRANSGENE (VIDT) IN THE AEDES AEGYPTI

Vivian Petersen - Department of Entomology and Nematology, University of Florida; **Alexius Dingle** - Department of Entomology and Nematology, Texas A&M University; **Zach N. Adelman** - Texas A&M University; **Bianca C. Burini** - Department of Entomology and Nematology, Florida Medical Entomology Laboratory

Introduction: Currently, more than 100 arboviruses are known to cause disease in humans, including dengue, Zika and yellow fever viruses and worldwide, one of the most important vector of those viruses is the Ae. aegypti. To control mosquitoes, the WHO recommends "integrated vector management" which involves the application of several strategies such as biological, chemical, mechanical, and genetic control. In this way, using a genetic approach, we propose the development of a mosquito, which dies upon contact with the virus. We have developed a genetic construct that contains a peptide neurotoxin (Scotox) from the scorpion, Androctonus australis Hector. After integration into the genome, the Scotox peptide will be expressed in the mosquito's nervous system but will be anchored in the endoplasmic reticulum. When flavivirus infection occurs, the viral protease will cleave a region that it recognizes in the transgenic constructions freeing Scotox, and killing the mosquito.

Materials and methods: Microinjection of the genetic construct was performed in the posterior pole of Aedes aegypti eggs of the Higgs strain. Surviving adult mosquitoes were placed in groups of 20-30 and crossed with uninjected mosquitoes. The embryos resulting from these crosses were analyzed under a fluorescence microscope to locate the larvae with the marker gene, which indicates that the insertion of the genetic construct was carried out successfully.

Results: We injected about 1,775 mosquito embryos and obtained a hatching rate of 18% and an adult mosquito survival rate of 87.5%. Around 11,986 larvae at the L3 and L4 stages were observed under a fluorescence microscope and 4 different strains were obtained (15 transgenic larvae in total) that expressed the marker gene, and we

obtained a transformation rate of 0.06. These 4 resulting lineages will be subjected to oral infection with DENV and ZIKV.

Conclusion: The microinjections performed on the embryos were successful and we expect that these transgenic lines generated will not survive after contact with the flaviviruses DENV and ZIKV.

70. ZIKA VIRUS'S IMPACT ON FETAL DEVELOPMENT: EXPLORING THE USE OF PORTABLE ULTRASOUND IN REINFORCING PUBLIC HEALTH SURVEILLANCE IN LOW RESOURCES SETTINGS

Rigan Louis - Department of Health Science, Emerging Pathogens Institute, College of Nursing, University of Florida; **Kevin Pierre** - Department of Radiology, College of Medicine, University of Florida; **Michael Mathelier** - Department of Medicine, College of Medicine, University of Florida; **Luke Trimmer-Smith** - Emerging Pathogens Institute, University of Florida; **Michael Weaver** - Department of Health Science, College of Nursing, University of Florida; **Dhanashree Rajderkar** - Department of Radiology, College of Medicine, University of Florida; **Derek Cummings** - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida; **Leslie Ann Parker** - Department of Health Science, College of Nursing, 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; **J. Glenn Morris, Jr.** - Department of Epidemiology, Emerging Pathogens Institute, College of Medicine, University of Florida

Introduction: Zika virus infection during pregnancy has been associated with severe birth defects, primarily microcephaly. This study aims to examine the relationship between Zika virus serologic status and fetal biometric measurements in a cohort of Haitian pregnant women, and the implication of the findings for public health.

Methods: From April 2018-August 2019, 74 of 216 (34%) pregnant women tested were seropositive for Zika virus using ELIZA or plaque reduction assays. Fetal biometric parameters (BPD, HC, AC, FL, and EFW) were obtained during antenatal visits. The deviations from expected

gestational age norms were reported. Descriptive statistics, independent t-tests, and MANOVA, were employed for analysis using SAS 9.4.

Results: Descriptive statistics showed variances in fetal parameters between the two serologic groups. Notably, the HC parameter showed a statistically significant reduction in Zika seropositive patients ($p = 0.0369$). However, other parameters did not display significant associations with seropositivity. Further multivariate analysis, such as MANOVA, indicated no significant difference in fetal parameter centroids between seropositive and seronegative groups.

Discussion: This study demonstrated an association between Zika seropositivity and reduced head circumference, aligning with previous findings on the impact of Zika virus. To our knowledge, this is one of the first investigations in Haiti to correlate fetal biometric parameters with Zika seropositivity, emphasizing the need for increased surveillance and preventive strategies in this region. The specificity of HC reduction may provide healthcare practitioners with a focused parameter to monitor at-risk pregnancies.

Conclusion: In this cohort of Haitian pregnant women, reduced fetal head circumference was associated with maternal seropositivity for Zika virus, pinpointing a critical biometric parameter for health care providers to monitor in at-risk pregnancy. Currently available and affordable portable ultrasounds devices and appropriate training for healthcare personal in resources limited settings, could contribute in both preventing pregnancy-related complications and identifying fetuses with potential Zika virus exposure.

NB: EFW (Estimated Fetal Weight), BPD (Biparietal Diameter), HC (Head Circumference), AC (Abdominal Circumference), Fl (Femur Length)

71. ABUNDANCE OF CORAL PATHOGEN VIBRIO CORALLIILYTICUS DOES NOT DRIVE DIFFERENCES IN SCTLD PRESENTATION

Allison Cauvin - Department of Natural Resources and Environment, College of Agricultural and Life Sciences, University of Florida; **Ellie Winer** - Department of Biology, College of Agricultural and Life Sciences, University of Florida; **Julie L. Meyer** - Department of Soil and Water Sciences, College of Agricultural and Life Sciences, University of Florida

Vibrio coralliilyticus is an emerging pathogen responsible for disease outbreaks in marine invertebrates, including coral. Additionally, co-infection with strains of V. coralliilyticus can exacerbate lesion progression and mortality in stony coral tissue loss disease (SCTLD). Therefore, we quantified the abundance of V. coralliilyticus in corals of varying resistance to SCTLD to determine its role in the dynamics of this disease in situ. The mountainous star coral Orbicella faveolata displays intraspecific variation in resilience to SCTLD, making it an ideal study species to investigate disease resistance drivers in coral. Ninety O. faveolata colonies from two regions along Florida's Coral Reef (FCR) were categorized a priori into levels of low, medium, or high resistance based on individual SCTLD history. A droplet digital PCR assay targeting the virulence factor VcpA, a zinc metalloprotease, was employed to quantify the presence of V. coralliilyticus in these corals. Preliminary analyses found no significant differences in the abundance of the vcpA gene of V. coralliilyticus between O. faveolata colonies across resistance categories or whether or not the coral ever became affected by SCTLD. Thus, V. coralliilyticus was not found to be driving intraspecific differences in resistance to SCTLD, nor was the amount of VcpA significantly different between corals which were affected or unaffected by SCTLD by the end of the study. V. coralliilyticus was absent or detected in very low abundances in most of the individuals throughout most of the study. This is in accordance with other studies that found V. coralliilyticus is not the primary pathogen of SCTLD. However, V. coralliilyticus abundances

peaked during the third sampling period, indicating potential seasonal shifts in the abundance of this bacteria. Future directions of this work include developing assays for the hydrogen cyanide synthase and LAP thiopeptide genes. The hydrogen cyanide production genes are additional virulence factors encoded in the V. coralliilyticus genome that will help further ground truth the abundance values calculated with the vcpA gene, while the LAP thiopeptide production genes have only been sequenced from V. coralliilyticus strains found in diseased corals and may better correlate with differences in SCTLD resilience. The results of this study will demonstrate the role of V. coralliilyticus in intraspecific variation in resistance to SCTLD.

72. ALTERED TLR INNATE IMMUNE SENSING OF PORPHYROMONAS GINGIVALIS VIA MICROBIAL SPHINGOLIPID SYNTHESIS

Zavier Eure - Department of Oral Biology, College of Dentistry, University of Florida; **Jose Solbiati** - Department of Oral Biology, College of Dentistry, University of Florida; **Frank Gibson III** - Department of Oral Biology, College of Dentistry, University of Florida

Background and Objectives: Periodontal disease is a chronic oral inflammatory disease with high prevalence in adults over the age of 30. Porphyromonas gingivalis (Pg) is a key microbe that is part of the subgingival microflora and is closely associated with periodontal disease. Our previous data show sphingolipids (SLs) found in Pg are transferred to host cells and limit host inflammatory responses to both Pg and its outer membrane vesicles (OMVs). Innate immune sensing by Toll-like receptors (TLRs) and downstream signaling molecules (i.e. MyD88) are known to influence Pg-elicited inflammation, however the contribution of SLs on innate immune sensing and reduced inflammation to Pg is unknown and is the primary goal of this study.

Methods: To investigate the contributions of SLs on TLR sensing and MyD88-dependent signaling, TLR2 and TLR4 reporter cells were challenged with Pg W83 wild type (WT) strain or a SL null mutant (SPT) (MOI:100) and OMVs (1000 particles/cell) purified from these strains.

Changes in MyD88 protein expression of Pg challenged THP-1 macrophages were determined via western blot analysis.

Results: TLR2 sensing of Pg and its OMVs were restricted by the presence of SLs. The presence of SLs in Pg promoted sensing via TLR4. In addition, Pg and its OMVs elicits MyD88-dependent NF-kB activation which was also limited by the presence of SLs. Interestingly, preliminary evidence supports limited MyD88 protein in Pg WT-treated THP-1 macrophages when compared to the □SPT mutant which displayed prominent MyD88 protein levels.

Conclusion: Our findings suggest SLs influences TLR sensing of Pg during live bacterial infection. In addition, TLR2 is a predominant sensor of Pg W83 OMVs and SLs dampen TLR2-mediated responses to OMVs. These data suggest that SLs are involved Pg-elicited immunomodulation via MyD88-dependent NF-kB activation and MyD88 degradation.

73. BURKHOLDERIA THAILANDENSIS EXPRESSING THE PATHOGENIC CAPSULAR POLYSACCHARIDE TRAIT IS DIVERSE THAN OUR THOUGHT

Apichai Tuanyok - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Pitchaporn Akaphan** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Pumin Sintara** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Pacharapong Khrongsee** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida; **Jedsada Kaewrakmuk** - Prince of Songkla University, Thailand

Introduction: Burkholderia thailandensis is a non-pathogenic bacterium typically found in soil and is not harmful to healthy individuals. It belongs to the Burkholderia pseudomallei complex (BPC) and is used as a safe model to study B. pseudomallei, the pathogen that causes melioidosis, mainly in tropical areas. Previously, certain strains of B. thailandensis have been identified to express the pathogenic capsular polysaccharide, also known as B. thailandensis Capsular Variant (BTCV). These strains

were believed to be narrowly divergent, primarily associated with the sequence type ST696 in Southeast Asia, or its close variant, ST101, in the southern United States.

Methodology: During the environmental survey of B. pseudomallei in southern Thailand, we used Ashdown's agar, a selective agar, and a latex agglutination assay targeting capsular polysaccharide (CPS) as a presumptive test for B. pseudomallei. These bacterial isolates were further tested by the TTS-1 assay, a real-time PCR assay specific to B. pseudomallei. If they were not B. pseudomallei, a series of real-time PCR assays developed in this study were used to identify if they were BTCV or other Burkholderia species. SDS-PAGE and Western blotting analysis were employed to identify the presence of B. pseudomallei CPS in BTCV isolates. Typical B. thailandensis and BTCV isolates were genotyped by multi-locus sequence typing (MLST) and further clustered with other known sequence types by goeBurst.

Results: We found that several bacterial isolates grown on Ashdown's agar were positive in the latex agglutination test but failed to amplify the TTS-1 PCR target. Multiple BTCV isolates were identified; they had ST696, similar to those previously found in other parts of Thailand, Cambodia, and Vietnam. However, one BTCV isolate had a new sequence type, ST2063, found in an area near the Malaysian border. Interestingly, ST2063 did not cluster with other BTCV strains that had ST696 and ST101, but instead grouped with typical B. thailandensis strains from Africa.

Conclusion: This was the first time that BTCV was discovered in southern Thailand. The discovery of ST2063, a new sequence type, has led us to believe that BTCV is more diverse than previously thought. Furthermore, through detailed genetic and phenotypic analyses, we have shown that these BTCV and other B. thailandensis strains are capable of competing with B. pseudomallei, likely through interactions involving phages.

74. DISTRIBUTED COMPACT PLASMA REACTOR STERILIZATION FOR PLANETARY PROTECTION AND CONTAMINATION CONTROL FOR SPACE MISSIONS

Tamara Revazishvili - Emerging Pathogens Institute, University of Florida; **Justin Kosky** - SurfPlasma, Inc; **Emma Mastro** - SurfPlasma, Inc; **Nona Rurua** - Emerging Pathogens Institute, University of Florida; **Davit Kobaidze** - Emerging Pathogens Institute, University of Florida; **Sarthak Roy** - SurfPlasma, Inc; **Sierra Brown** - SurfPlasma, Inc; **Vladimir Vichnyakov** - SurfPlasma, Inc; **Subrata Roy** - College of Engineering, University of Florida

This work presents a proof-of-concept study performed to establish the effectiveness of the Active Plasma Sterilizer (APS) and evaluates it for sterilization applications pertaining to planetary protection. The SurfPlasma Active Plasma Sterilizer (APS) is a compact, energy efficient, and portable sterilization system for use with a variety of materials. The system consists of patented SurfPlasma low power, compact plasma generators combined with an airtight testing 1 cubic foot testing chamber. The APS uses these plasma generators to ionize the air inside the testing chamber, producing reactive oxygen and nitrogen species. These species, which include ozone, kill surface pathogens present inside the chamber. Testing has shown capabilities to kill a large variety of pathogens, from gram-positive and gram-negative bacteria to viruses and fungi, in timeframes of 45 minutes or less. During the testing period, a testing prototype version of the APS chamber was used.

Sterilization tests the efficacy of the Active Plasma Sterilizer (APS) prototype in sterilizing surfaces were performed containing 2 pathogenic bacteria: Geobacillus stearothermophilus and Deinococcus radiodurans (both gram-positive bacteria); and fungi - Aspergillus fumigatus on Aluminum material, relevant to space missions.

Results show that the APS can achieve 100% - full killing of the two species - Deinococcus radiodurans within 25 minutes and Aspergillus fumigatus within 35 minutes on selected material; and 4 to 5 log

reductions of the Geobacillus stearothermophilus, within 35 minutes at 6 points inside the APS.

This study shows the potential of the APS as a sterilization technology for planetary protection applications with advantages of uniform spatial decontamination, low processing temperatures, low exposure times, lightweight design with no moving parts, ability to decontaminate porous surfaces and compatibility with space mission relevant materials.

75. EFFECT OF EARLY AND ADEQUATE EMPIRIC ANTIBIOTIC THERAPY FOR HOSPITAL-ACQUIRED SEPSIS IN THE AGE OF ANTIMICROBIAL RESISTANCE: A TARGET TRIAL EMULATION USING MACHINE LEARNING

Scott Cohen - 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; **Sarah Ser** - Department of Epidemiology, College of Public Health and Health Professions, 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: Antimicrobial resistance (AMR) in hospital-acquired (HA) sepsis underscores the urgency for timely and effective antibiotic strategies however, the inherent delays in culture results make empiric antibiotic selection challenging. To better understand the burden and prognostic impact, this study leveraged target trial emulation and machine learning to estimate causal effects of early and adequate antibiotic therapy in HA-sepsis, as well as the moderating role of AMR.

Methods: We examined approximately 140,000 hospital encounters with documented bacterial infection from January 2010 to May 2023 at a single tertiary-care hospital. Those with hospital-acquired sepsis defined using the CDC's Adult Sepsis Event criteria were included. Treatment groups were classified based on the initiation of adequate or inadequate empiric antibiotics within the first 6, 12, 18, 24, 36, and 48 hours after blood culture collection, with the primary outcome being 30-day all-cause

mortality. Empiric antibiotic adequacy was determined using in vitro susceptibility test report. Average treatment effects (marginal hazard ratio) of adequate therapy were estimated using marginal structural Cox proportional hazards models. Inverse probability weighting (IPW) was estimated using machine learning methods including logistic regression, lasso regression, gradient-boosted decision trees, random forest, and ensemble learning.

Results: Among 49,581 hospitalizations, 2019 (4%) met the criteria for HA-sepsis and were included. After assessing antibiotic-pathogen characteristics, 770 (38%), 559 (28%), 458 (23%), 377 (19%), 323 (16%), and 294 (15%) received inadequate empiric antibiotic therapy at 6, 12, 18, 24, 36, and 48 hours after blood culture collection, respectively. Patients receiving inadequate antibiotic therapy were more likely to have Acinetobacter species ($p=0.002$), Enterococcus species ($p<0.001$), and Pseudomonas aeruginosa ($p<0.001$) isolated compared to those receiving concordant antibiotics. Marginal hazard ratio of adequate antibiotics at 24 hours on 30-day all-cause mortality was 0.72 (95%CI: 0.60-0.88) and 0.65 (95%: 0.52-0.82) for unadjusted and IPW models. Treatment effects increased up to 24 hours after blood culture collection (P for Trend=0.019). Conditional average treatment effect of adequate antibiotics at 24 hours among those with Escherichia coli was 0.44 (95%CI: 0.28-0.72). AMR classes (e.g. MRSA, ESBL, VRE, and CRE) did not modify treatment effects.

Conclusion: Approximately one in five patients with HA-sepsis received inadequate empiric antibiotic therapy and delays in adequate antimicrobial treatment up to 24 hours after blood culture collection caused increased mortality. Our findings identify specific infections most likely to benefit from emerging diagnostic technologies. Future research will further assess heterogeneity of treatment effects across patient characteristics.

76. ENSO VARIABILITY AND VIBRIOSIS DYNAMICS: UNRAVELING THE CLIMATE-HEALTH NEXUS IN COASTAL REGIONS OF FLORIDA

Komalpreet Singh - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Moiz Usmani** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Antarpreet Jutla** - Department of Environmental Engineering Sciences, College of Engineering, University of Florida

The imperative for comprehensive research into the widespread presence of vibrios, associated with seafood-related human illnesses, is highlighted, particularly in the coastal waters of Florida. Despite the prevalence of vibrios, the impact of El Niño Southern Oscillations (ENSO) on their dynamics remains insufficiently explored. This study seeks to unravel the intricate relationships between vibrios and ENSO patterns. Robust statistical correlations between the variables were established using the Kendal Tau and Mann-Kendall tests. Vibrio cases across Florida were categorized into eight zones for a nuanced understanding. The Oceanic Niño Index (ONI) during La Niña events showed a significant ($p<0.05$) correlation with vibrio clinical cases, specifically in the East Central zone during June-July-August ($r=0.89$). Conversely, El Niño exhibited a correlation during the same months but for the West Central zone, extending into September-October-November for the Northeast zone ($r=0.82$). While identifying clear trends in El Niño and La Niña proves challenging, the months of March-April-May ($r=0.49$), June-July-August ($r=0.49$), and September-October-November ($r=0.53$) witnessed a substantial increase in vibriosis cases across Florida, excluding Vibrio Vulnificus cases. Notably, during December-January-February, upward trends were observed in all regions for vibriosis ($r=0.46$), except for Northern Florida. Interestingly, the cases of Vibrio Vulnificus exhibited a significant but weak increase in more zones of Florida during June-July-August ($r=0.22$). This intricate interplay between the two variables serves as a compelling impetus to further explore an ecological approach, aiming for a more profound understanding.

77. EVALUATING LIVESTOCK COMMINGLING IN AREAS OF SUSTAINED BRUCELLOSIS RISK IN KAZAKHSTAN

Aryn O'Dell - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Nurkuisa M. Rametov** - Research Institute for Biological Special Problems, Otar, Kazakhstan; **Mukhit Orynbayev** - Research Institute for Biological Special Problems, Otar, Kazakhstan; **Syrym Kopeyev** - Research Institute for Biological Special Problems, Otar, Kazakhstan; **Aslan Kerimbayev** - Research Institute for Biological Special Problems, Otar, Kazakhstan; **Talgat Karibayev** - National Reference Veterinary Center, Astana, Kazakhstan; **Maxat Berdikulov** - National Reference Veterinary Center, Astana, Kazakhstan; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Jeremy P. Orange** - Department of Geography, Emerging Pathogens Institute, 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

Brucellosis is a zoonosis caused by species of Brucella bacteria. The disease is global with heavy burden in underdeveloped countries. Kazakhstan, in Central Asia, reports high human/livestock burden, requiring high costs for animal testing and slaughter. Brucella spp. show relatively high host affinity (e.g., Brucella melitensis in sheep and B. abortus in domestic cattle) and vaccines are Brucella spp. specific. Additionally, the risk of brucellosis transmission between commingling livestock species and from livestock to wildlife is poorly understood. This necessitates disease surveillance and study of brucellosis ecology, such as commingling, to better understand the ecology of brucellosis in Kazakhstan. The primary objective of this study was to determine the degree of commingling between four livestock species (sheep, goats, cattle, horses), and determine commingling between livestock and wildlife (Roe Deer [Capreolus capreolus]). Motion-triggered cameras were deployed on four privately owned farms in Kazakhstan from 2018 to 2021. There, farms represent areas of sustained Brucella transmission in

southern Kazakhstan. Here we assimilated data from several ongoing and published studies to map this ecology. For camera work, all images were tagged with species/behavior observations to create kernel density estimates of species-specific diel activity patterns and plots of commingling percentages between sheep and other livestock species. We found overlaps in diel activity for most livestock (sheep, goats, cattle, horses). Livestock showed three grazing peaks: morning, afternoon, and evening. Roe deer were crepuscular. High levels of direct commingling occurred between sheep and domestic cattle: 18.18% of the time sheep grazed, only cattle grazed with them; ~28% of the time sheep were grazing, cattle and other livestock also grazed. Molecular evidence from ongoing and previous studies suggests possible shared Brucella spp. in both sheep and domestic cattle and high rates of livestock Brucella species from both hosts in our study area. This indicates that interactions between both species are likely for brucellosis transmission in shared grazing patches. Sheep were also found to directly commingle with all livestock species in the study. Additionally, while livestock were not seen directly commingling with wild cervids, there was evidence of shared patch use between domestic cattle and horses with deer in forested farms within the study area. These data will aid policy makers in the identification of effective and economically efficient intervention strategies to lessen the burden of brucellosis to Kazakhstan and other endemic disease foci.

78. EVOLUTION OF THE BACTERIAL SPOT PATHOGEN XANTHOMONAS PERFORANS ON TOMATO AND PEPPER

Jeannie M. Klein-Gordon - Department of Plant Pathology, University of Florida; **Aastha Subedi** - Department of Plant Pathology, University of Florida; **Sujan Timilsina** - Department of Plant Pathology, University of Florida; **Gerald V. Minsavage** - Department of Plant Pathology, University of Florida; **Pamela D. Roberts** - Department of Plant Pathology, University of Florida; **Gary E. Vallad** - Department of Plant Pathology, University of Florida; **Jeffrey B. Jones** - Department of Plant Pathology, University of Florida; **Erica Goss** - Department of Plant Pathology, Emerging Pathogens Institute, University of Florida; **Apekshya Parajuli** - Department of Plant Pathology, University of Florida

Introduction: Xanthomonas spp. that cause bacterial spot of tomato and pepper have been important model systems for understanding molecular interactions between crop plants and bacterial pathogens. Extraordinary diversity in Xanthomonas perforans makes it an ideal model for understanding the evolution of emerging bacterial plant pathogens. We previously documented gain and loss of Type III effectors (T3E) in X. perforans on tomato in Florida and identified those that might be targets for resistance breeding. Since that time, we have documented additional diversity in the X. perforans populations in Florida. Here, we examined 281 X. perforans strains collected from tomato in 2017 and 35 from pepper collected from 2019-2021 for T3E content and variation.

Methods: We generated draft genomes for X. perforans strains collected from tomato and pepper. The presence of 72 T3Es were investigated for all genomes using tBLASTn. Putative sequences of effectors were aligned to examine nucleotide variation. TAL effector was identified using PCR amplification of the repeat region.

Results: Thirty-two Type III effectors were found in 281 X. perforans strains collected from tomato fields in Florida in 2017. The results show gain of effectors (e.g., xopL, xopAQ), possibly via introduction of new genotypes of X. perforans, and loss of effectors from some strains (e.g., xopJ4, xopP). Some alleles appeared nonfunctional due to a missing or

premature stop codon, including alleles for XopP, XopAD, and XopAF. Eight effectors were conserved in all strains with only a single allele (AvrBs2, XopR, XopX, XopAE, XopAR, XopAU, XopAZ). On pepper in Florida, an emerging lineage of X. perforans is closely related to tomato strains found in other locations and contains TAL effector AvrHah1. Genetically similar strains that were isolated from tomato were pathogenic on pepper and grew to the same population sizes in pepper leaves as strains isolated from pepper. Strains with avrHah1 produced rapid necrosis.

Conclusions: New genetic diversity introduced into Florida, USA has brought in new effectors and genetic variation in existing effectors. In addition, some effectors that were previously conserved among strains have been lost. An aggressive lineage of strains previously found on tomato and pepper in Alabama, USA is emerging in pepper production in Florida. A survey of the genetic diversity of effectors in X. perforans is being used to identify conserved effectors and screen for novel host resistance against X. perforans.

79. EXPLORATORY SPATIAL DATA AND PHYLOGENETIC ANALYSIS OF BACILLUS ANTHRACIS DIVERSITY WITHIN AND BETWEEN OUTBREAKS ACROSS NORTHERN VIETNAM, 2008 – 2023

Morgan Metrailer - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Van Khang Pham** - Vietnam National Institute of Hygiene and Epidemiology; **Tan Luong** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Minh Hoa Luong** - Vietnam National Institute of Hygiene and Epidemiology; **Quang Thai Pham** - Vietnam National Institute of Hygiene and Epidemiology; **Nhu Duong Tran** - Vietnam National University; **Michael Norris** - University of Hawai'i; **Jose Miguel Ponciano** - Department of Biology, University of Florida; **Thi Thu Ha Hoang** - Vietnam National Institute of Hygiene and Epidemiology; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Zoonotic diseases are a major public health concern, particularly in rural areas where human-animal interactions are common and adequate healthcare is limited. Anthrax, caused by the bacterium Bacillus anthracis, is a potentially fatal zoonotic disease, often affecting herbivories (livestock and wildlife) with regular spillover into human populations. It is a primary zoonosis of concern in Vietnam, following a national circular in 2016, with many reported cases occurring in six northern Vietnam provinces. Here we investigated the spatial and temporal patterns of anthrax outbreaks within these provinces from 2008-2023, utilizing coupled epidemiological and molecular methodologies for strains from 2019 - 2023. In recent years the surveillance and collection methods have expanded, leading to improved epidemiological traceback of anthrax cases and increased sampling of the environment and animal hosts. In 2023, clinical cases of anthrax were reported in 4/6 northern provinces; of which, B. anthracis strains were successfully isolated from human, animal, and environmental samples in 3/6 provinces. Isolated samples were whole-genome sequenced and genetic typing methods, including single-nucleotide polymorphism (SNP) typing and 25-marker multi-locus VNTR assays (MLVA), were used to investigate linkages between provinces, communes (sub-districts), and villages. Genetic similarity between isolates at varying distances was investigated using the Tau statistic, identifying a peak of high similarity at lower distances (within/between villages) as well as a second peak at greater distances (>50 km). At local levels, high genetic similarity supports the transmission from local environmental B. anthracis reservoirs to animals, with subsequent spillover to humans. The sporulating nature of B. anthracis is considered when shared genotypes are observed over greater distances as prevalent genotypes may persist in the soil. Alternatively, there are outbreaks where the phylogenetic and epidemiological findings support the hypothesis that the movement of genotypes is associated with animal slaughter or meat movement. Often, these situations are exasperated by the financial burden faced by individuals or communities who regularly experience outbreaks. Preventative vaccination campaigns, based on timing revealed by epidemiological analyses, and efficient carcass management is highly suggested in communities that regularly

experience outbreaks, likely from local sources. Understanding the distribution of anthrax cases throughout the region and their phylogenetic relationships can inform knowledge campaigns regarding animal butchering, meat sale (or re-sale), and safe practices within meat markets.

80. EXPLORING THE ECOLOGICAL RAMIFICATIONS OF ANTIBIOTIC INTERVENTION IN CITRUS RETICULATA THROUGH ITS EFFECTS ON RHIZOSPHERE BACTERIAL COMMUNITIES AND METABOLITES

Toi Ketehouli - Department of Plant Pathology, 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; **Samuel J. Martins** - Department of Plant Pathology, College of Agricultural and Life Sciences, University of Florida

Citrus greening disease, caused by the bacterium Candidatus Liberibacter asiaticus, poses a substantial threat to global citrus production. Antibiotics like streptomycin (Str) and oxytetracycline (Otc) have been used as possible management techniques to reduce the effects of the disease. Nevertheless, the ecological ramifications of these antibiotic treatments on the rhizosphere, a crucial area for interactions between plants and microorganisms, are still poorly understood. This study examined the effects of Str and Otc on the bacterial communities and metabolites in the rhizosphere of Citrus reticulata. We explored the emergence and persistence of antibiotic-resistant bacteria (ARB) in the citrus rhizosphere through bacterial cultivation and colony-forming unit (CFU) measurement. To elucidate the changes in the composition and diversity of the rhizosphere bacterial community in reaction to antibiotic treatments, we employed high-throughput sequencing of 16S rRNA genes. Metabolomic investigation of the citrus rhizosphere examined subsequent changes in metabolic profiles. The ARB cultivation results showed that the concentration of bacteria (colony-forming unit) in all antibiotic treatments decreased. The 16S rRNA gene-based microbiome analysis showed significant alterations in the relative abundance of specific bacterial taxa, particularly the Pseudomonas and Streptomyces genera, in response to both Str and Otc treatments. Metabolomic

investigations demonstrated that the top ten downregulated and upregulated metabolites between streptomycin- and oxytetracycline-treated citrus plants were entirely different, suggesting that these two antibiotics may have different plant/microbe targets or induce different stress responses. Gaining a comprehensive understanding of these interactions is essential for formulating effective and sustainable approaches to address citrus greening disease while limiting any unintentional harmful effects on the ecological balance of the citrus rhizosphere. This study enhances the overall understanding of antibiotic-based therapies for controlling plant diseases and their impact on the plant microbiome and health.

81. FRANCISELLA SECRETES PDPE THROUGH THE TYPE VI SECRETION SYSTEM TO INHIBIT HOST CELL DEATH

Nicole Adams - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Danielle Hulvey** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Nimra Khalid** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Alexandra Burne** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Mary Brown** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Aria Eshraghi** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida

Francisella tularensis is the causative agent of the fatal zoonotic disease tularemia and poses a major threat to global public health. This virulent Gram-negative bacterium is highly infectious, requiring fewer than 10 viable bacteria to establish infection in humans. F. tularensis requires a unique contractile toxin secretion apparatus, known as the type VI secretion system (T6SS), to invade host cells, proliferate, and cause disease. Although the T6SS has been studied for over 15 years, we have yet to characterize the full repertoire of the substrates that it injects into host cells. In this project, we took a comprehensive approach to query the genomic locus in Francisella that encodes the T6SS and discovered that PdpE is a secreted toxin. Western blot analyses revealed that PdpE is secreted in a T6SS-dependent fashion, and it interacts with another

secreted toxin that is critical for pathogenesis. To determine the role of PdpE in Francisella virulence, we infected cultured murine macrophages with a ΔpdpE mutant. Unexpectedly, compared to wild type, the deletion mutant caused increased macrophage toxicity and secretion of IL-1β, and expression of PdpE in this mutant restored these phenotypes. In vivo studies in Galleria mellonella larvae showed more rapid death in those infected with the ΔpdpE mutant, indicating greater pathogenicity in the absence of PdpE. Taken together, these data indicate that PdpE is a secreted toxin that controls host cell viability, and this research has provided new insights into host-Francisella interactions.

82. GENETIC EVOLUTION OF VIBRIO PARAHAEMOLYTICUS AND VIBRIO VULNIFICUS FROM AQUATIC BIRDS AND SEAWATER RESERVOIRS IN THAILAND.

Juan Guillermo Perez Jimenez - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida

Background: Vibrio vulnificus and Vibrio parahaemolyticus are naturally occurring inhabitants of estuarine and marine environments worldwide, responsible for a high number of deaths due to undercooked seafood consumption all over the world. Aquatic birds and seawater in Thailand are reservoirs for these bacteria, and it has been shown that migratory birds widely carry V. parahaemolyticus by consuming marine products. Therefore, they are a cause of the spread of these bacteria worldwide. The objective of this study is to apply a phylogeography framework to reconstruct the spatial and temporal dynamics of V. parahaemolyticus and V. vulnificus from an estuarine area in the province of Samut Prakan, Thailand.

Methods: We used Whole Genome Sequencing (WGS) data from 50 isolates from seawater and two isolates from the dominant aquatic bird, Brown-headed gull (Chroicocephalus brunnicephalus), during nine months (August 2016 - April 2017) in the Bang Pu recreational center, Samut Prakan, Thailand. WGS, mapping, and high-quality single-nucleotide polymorphism calling were performed for the isolates. A

phylogeography analysis was conducted for 52 isolates from the estuarine area including 187 V. parahaemolyticus and 43 V. vulnificus complete genomes sequences from SRA. A maximum likelihood phylogenetic tree was analyzed with IQ-TREE, and Bayesian ancestral state (phylogeography) tree reconstruction were performed with BEAST version 1.10.4 software.

Results: WGS data allowed us to confirm the widespread presence of V. parahaemolyticus and V. parahaemolyticus in aquatic birds and seawater in the province of Samut Prakan, Thailand. This study revealed compelling evidence supporting the role of migratory birds as carriers of V. parahaemolyticus through their consumption of marine products. The temporal scope from August 2016 to April 2017, allowed a comprehensive understanding of the evolutionary dynamics of these bacterial species. The phylogenetics analysis shows high genetic diversity for both vibrios, which are divided into separate lineages. Also, environmental isolates from seawater and aquatic birds are divided within each lineage.

Conclusion: V. parahaemolyticus and V. vulnificus are present in aquatic birds and seawater, and they are genetically unrelated. Thus, it is necessary to study more isolates and compile more basic information about these enteric bacterial infections. Next, we will focus on investigating antibiotic-resistant genes in V. parahaemolyticus and V. vulnificus from birds and water sources, an urgent need as the evolutionary mechanisms of resistance are still unclear.

83. IMAGING AND CLINICAL CHARACTERISTICS OF CEREBRAL NOCARDIOSIS: A SINGLE INSTITUTIONAL RETROSPECTIVE REVIEW OF 26 CASES

Brandon Lucke-Wold - Department of Neurosurgery, College of Medicine, University of Florida

Introduction: Nocardiosis is a rare opportunistic bacterial infection with various clinical manifestations, including CNS involvement in the form of multiple brain abscesses. Clinical knowledge of this entity is important given the increasing use of medical therapies which produce immune suppression and because many of its features may be nonspecific and misdiagnosed in context of other underlying comorbidities.

Methods: This retrospective single institutional review analyzed the imaging, clinical, and microbiological characteristics of proven cerebral Nocardiosis cases in 26 patients over approximately 19 years.

Results: Imaging features generally showed multiple small ring-enhancing lesions throughout the brain with exuberant perilesional edema and central diffusion restriction. Clinically, patients were often immunocompromised, but a few were immune competent. The study identified various Nocardia subspecies with different antibiotic susceptibilities. Many of our patients required neurosurgical intervention for definitive diagnosis.

Conclusions: This is one of the largest single institutional studies of the clinical and imaging features of CNS Nocardiosis in the world literature. The study emphasizes the importance of aggressive diagnostic intervention (including neurosurgical biopsy). This is paramount given the increasing use of immunomodulatory therapies and rising number of somatic allograft transplants requiring long-term immune suppression.

84. MODELING THE EFFECTS OF VACCINE INTERVENTIONS ON LIVESTOCK ANTHRAX OUTBREAK DYNAMICS IN A NORTHERN PROVINCE OF VIETNAM

Francisca Javiera Rudolph - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Tan Luong** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Minh Hieu Tran** - Lao Cai Provincial Center for Disease Control, Lao Cai City, Lao Cai province, Vietnam.; **Ba Uyen Pham** - Lao Cai Provincial Sub-Department of Animal Husbandry and Animal Health, Lao Cai City, Lao Cai province, Vietnam.; **Van Khang Pham** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.; **Thanh Long Pham** - Department of Animal Health, Ministry of Agriculture and Rural Development, Hanoi, Vietnam.; **Quang Thai Pham** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.; **Jose Miguel Ponciano** - Department of Biology, College of Liberal Arts and Sciences, University of Florida; **Juan Pablo Gomez** - Department of Chemistry and Biology, Universidad del Norte, Barranquilla, Colombia.; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: Anthrax is an underreported zoonosis primarily affecting herbivorous animals, caused by Bacillus anthracis, an environmentally mediated, spore-forming pathogen. Spores can survive in the soil environment for years before infecting other animal individuals. In Vietnam, anthrax has been concentrated in northern provinces where limited transportation has created challenges for disease control, including livestock vaccination implementation. In contexts where data are incomplete, mathematical modeling with computational simulations can provide a powerful means of studying anthrax dynamics. The SMILE model successfully characterizes anthrax outbreaks in the absence of vaccine interventions in Montana. Here, we modified SMILE to incorporate livestock vaccine coverage and used the vaccine-modified model to characterize buffalo anthrax outbreak dynamics in Lao Cai province, Vietnam.

Methods: SMILE employs a compartmental modeling approach considering five anthrax disease states: Susceptible (S – naive individuals with no infection immunity), Infected (I – actively infected individuals), Immune (M – individuals survive infection), Local infectious zone (L – individuals do not survive the infection), and Environment (E – spores released from a LIZ to the soil). We extended the original model by modifying the rates at which individuals survived the infection and moved into the Immune compartment. Starting with a deterministic model, simulated the effects of host population dynamics and seasonal forcing under scenarios with and without vaccination testing a range of vaccination levels. Additionally, we compared simulated data with provincial surveillance data from 1991 to 2013 to evaluate model prediction accuracy.

Results: We examined the relative roles of seasonal forcing and population dynamics in producing outbreaks under scenarios with and without vaccination. When seasonal forcing was ignored, vaccination allowed the susceptible population to recover, and smaller outbreaks occurred relative to decreases in vaccination rates. Under scenarios with seasonal forcing, anthrax dynamics followed seasonal transmission with large initial outbreaks that exhausted the susceptible host pool. When incorporating vaccination into the scenarios with seasonal forcing, disease outbreaks closely matched the timing of seasonal transmission, but the magnitude of outbreaks was dependent on the levels of vaccination.

Conclusions: Our SMILE extension and these model outputs highlight the capabilities of using a simulation approach to understand anthrax outbreak dynamics and the effects of vaccination in modifying the timing and magnitude of outbreaks. This framework can provide some basic guidelines for vaccination levels that can reduce the number and magnitude of anthrax outbreaks in livestock.

85. MULTISYSTEMIC DISEASE AND SEPTICEMIA CAUSED BY BURKHOLDERIA CEPACIA COMPLEX IN AN AMERICAN QUARTER HORSE (EQUUS CABALLUS)

Jason Thornton - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **John Roberts** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Apichai Tuanyok** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida

Introduction: The Burkholderia cepacia complex (Bcc) is a heterogeneous group of Gram-negative rod-shaped bacteria from the Burkholderia genus that typically cause opportunistic infections in immunocompromised hosts. Outbreaks of disease often occur in the hospital setting via contaminated medical devices or in patients with chronic respiratory diseases such as cystic fibrosis. Bcc infections are often refractory to antibiotic treatment, and the disease can progress to life-threatening septicemia. A recent increase in case reports in healthy humans and animals suggests Bcc may be an emerging pathogen of concern.

Method: The tissue was fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections of the mass were stained with hematoxylin and eosin. Histochemical stains were performed to screen for bacteria and fungi.

Results: An 8-year-old quarter-horse gelding (Equus caballus) presented to the University of Florida Veterinary Medical Center with the chief complaint of a left retropharyngeal abscess. Clinical examination and radiographs revealed a well-circumscribed 20x10x18 cm round soft tissue mass caudal to the ramus of the mandible with displacement of the guttural pouch. Other exam findings included diffuse interstitial pneumonia, multiple cutaneous ulcers on the dorsal midline, anterior uveitis in the right eye, enlarged mesenteric lymph nodes, and suspected aneurysm of the right renal artery. Due to a declining clinical condition, the animal was humanely euthanized. Post-mortem aerobic culture of the retropharyngeal and mesenteric lymph nodes revealed pure growth of

Burkholderia cepacia. This finding was confirmed with a fatty acid analysis of the bacterial isolate.

Conclusions: This report describes the histomorphologic identification, culture, and characterization of an unusual case of Bcc in a horse. This case underscores the potential pathological impact of Bcc in horses, emphasizing the need for increased awareness and understanding of its emergence as a potential pathogen in diverse species.

Disclaimer: The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or U.S. Government.

86. NOVEL BACTERIOPHAGE COCKTAIL ELIMINATES PSEUDOMONAS AERUGINOSA IN VIVO AND REVEALS BACTERIAL GENES IMPORTANT FOR PHAGE INFECTION

Garrett L. Ellward - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Michael Bucher** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Ting Liu** - Emerging Pathogens Institute, University of Florida; **Sisse Diakite** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Rosanna Marsella** - Department of Small Animal Clinical Sciences, College of Veterinary Medicine, 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; **Kartikeya Cherabuddi** - Department of Medicine, College of Medicine, University of Florida; **Kwangcheol Casey Jeong** - Emerging Pathogens Institute, University of Florida; **Daniel Czyz** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Antimicrobial resistance (AMR) is a growing global issue attributed to millions of deaths worldwide and is responsible for crippling economic

burdens. In 2019 alone, AMR was attributed to 1.2 million deaths and associated with millions more. It has been estimated that, by 2050, if no alternative to failing treatments is implemented, AMR could be responsible for 10 million deaths each year, further breaking down medical infrastructure worldwide. Pseudomonas aeruginosa, a gram-negative bacterium found ubiquitously in the environment, typically infects individuals with cystic fibrosis, burn wounds, or compromised immune systems. In 2019, P. aeruginosa was one of six pathogens responsible for 929,000 (~73%) of deaths attributable to AMR. Intrinsically resistant to most antimicrobials and a common nosocomial infective agent, P. aeruginosa is listed as a critical threat by both the Centers for Disease Control and Prevention and the World Health Organization. Bacteriophages (phages), viruses that target and kill bacteria during replication, are a proposed alternative to antibiotics. Using a high-throughput screening pipeline, seven novel bacteriophages were isolated from wastewater systems, characterized, and tested against a library of 136 unique, clinically relevant isolates of Pseudomonas aeruginosa. Isolated bacteriophages showed strong lytic activity against Pseudomonas and could kill multiple pan-drug resistant strains. In a cocktail combination, isolated phages inhibited 75% (103/136) of P. aeruginosa isolates in vitro. In vivo experiments revealed that phages significantly reduced bacterial load across organs and protected mice against lethal bacteremia, resulting in phage-treated mice being cleared of clinical signs of infection 36 hours post-inoculation. Finally, using a genome-wide bi-direction BLAST comparing phage-sensitive and phage-resistant Pseudomonas aeruginosa isolates, 20 bacterial genes important for phage infection were identified and confirmed using strains from a comprehensive knockout library of non-essential Pseudomonas genes. Thus far, the cocktail shows strong clinical promise and identification of phage infection-relevant genes will lead to development of phage cocktails more effective towards specific bacterial AMR infections.

87. ONE HEALTH ON THE EDGE: RESULTS FROM A NETWORK ANALYSIS OF POLICIES GOVERNING HUMAN HEALTH AND ANIMAL HEALTH PLANNING IN VIETNAM

Tan Luong - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Thi Lan Phuong Nguyen** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Duc Phuc Pham** - Hanoi University of Public Health, Hanoi, Vietnam; **Thi Thu Ha Hoang** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Quang Thai Pham** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Nhu Duong Tran** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Duc Anh Dang** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Yujie Hu** - Department of Geography, College of Liberal Arts and Sciences, University of Florida; **Thi Mai Hung Tran** - National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; **Jason Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: One health is defined as an integrative and systemic approach to health that aims at optimizing the health of humans, animals, and shared environments. In Vietnam, a national One health circular number 16/2013/TTLT-BYT-BNNPTNT was jointly established in 2013 by the human and animal health sectors for controlling several prioritized zoonoses nationwide. The policy contributed significantly to Vietnam's COVID-19 pandemic control as both sectors cooperatively provided workforce and diagnostic resources. However, no studies have quantified the degree of importance of the One health policy in consideration of other health policies. Here, we employed network analysis methods to quantify the importance of Circular 16 in a zoonosis-related policy network. We also aimed to detect network communities that demonstrate policy silos between the health sectors.

Methods: A policy is controlled by a legal framework of other policies, in which a new policy is required to cite relevant policies established earlier. A policy citation creates a connection between two policies. Here, each policy is a network node, and each citation is a network edge. Network

structures were visualized using different functions of Gephi. In-degree and betweenness centrality were used to identify important policies. A node with higher centrality values is more important as the policy is cited more frequently (in-degree), and/or it connects other policies (betweenness). Constant Pott Model Leiden algorithm was employed for network community detection. If a silo is present, the human health and animal health policies will form separate network communities.

Results: Here, the policy network was formed by 296 policies (nodes), which were connected by 725 citations among the policies (edges). Circular 16 was among the five nodes with the highest in-degree (it was cited frequently) and betweenness centrality (it bridged other policies from both health sectors), indicating the importance of Circular 16 in the policy network. The community detection algorithm revealed siloing between the two health sectors' policies as they formed two major network communities. Our results demonstrate the importance of circular 16 in maintaining the intersectoral connections, and the vulnerability of the connections when circular 16 is outdated.

Conclusions: Here, we applied network analysis methods to evaluate a national health policy in Vietnam. The findings advocate for actions to maintain the national One health policy, establish more intersectoral connections at lower administrative levels, and change the silo structure of the policy network.

88. POPULATION GENOMICS OF XANTHOMONAS EUVESICATORIA OF PEPPER IN SOUTHWEST FLORIDA

Aastha Subedi - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Gerald V. Minsavage** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Jeffrey B. Jones** - Department of Plant Pathology, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Pamela D. Roberts** - 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

Bacterial leaf spot is one of the significant diseases challenging pepper growers globally and is primarily caused by Xanthomonas euvesicatoria (Xe). An extensive genomic study of this pathogen is limited which hinders a more precise estimate of population structure and diversity. The whole genome sequences of 103 Xe strains isolated from pepper from seven fields across Southwest Florida between 2019-2021 were used to investigate population genomic divergence, evolution, and variation in type III secretion effectors (T3SEs). Biochemical characterization showed that one-third of Xe strains were positive for amylase activity, a trait not typically observed in Xe. Phylogenetic analysis of core genomes revealed two distinct genetic lineages that corresponded to amylase activity. A comparison of amylolytic and non-amylolytic strains revealed that all nonamylolytic strains contain a single base pair frameshift deletion in the alpha-amylase gene. Molecular clock analysis dated the emergence of amylolytic strains to approximately 1985 and was the only clade among many Xe strains. Analysis of T3SEs revealed variation in four effectors including mutations in avrBs2, resulting in the inability of those strains to elicit the hypersensitive response in pepper plants containing Bs2. Based on this study, it is important to forecast the emergence of such strains, identify the genetic factors that could be

targeted for novel disease management interventions as well as to further investigate population structure, diversification, and evolution of this economically important pathogen.

89. SEQUENCING OF PHOSPHOPEPTIDE ANIONS VIA SEQUENTIAL CHARGE INVERSION ION/ION REACTION AND ELECTRON CAPTURE DISSOCIATION

David V. Donndelinger - Department of Chemistry, University of Florida; **Tingting Yan** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida; **Troy R. Scoggins IV** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida; **Boone M. Prentice** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida

Protein phosphorylation is a key post-translational modification (PTM) common in biological systems. Changes in the phosphoproteome caused by host-pathogen interactions, such as with Clostridioides difficile, alter host gene expression and modify innate immune adaptor proteins requiring comprehensive analytical methods to investigate these phosphorylation-mediated mechanisms. The sequence of a peptide, including the presence and position of PTMs is critical to understanding its structure and function. Phosphopeptides readily ionize as singly charged anions in negative ion mode using matrix-assisted laser desorption/ionization (MALDI). However, traditional peptide sequencing using collision induced dissociation (CID) induces the loss of labile phosphate groups, preventing PTM localization. Conversely, electron capture dissociation (ECD) preserves phosphate bonds and allows for full peptide sequencing yet ECD necessitates a multiply charged precursor cation. Gas-phase charge inversion ion/ion reactions are employed here to convert the charge state of a peptide following ionization, but prior to ECD fragmentation. We demonstrate transformation of MALDI-generated phosphopeptide anions into dications via a gas-phase charge inversion ion/ion reaction.

A 7T solariX FT-ICR mass spectrometer (Bruker Daltonics) was used for all gas-phase charge inversion ion/ion reactions, ECD, and CID experiments.

Briefly, phosphopeptide anions generated via MALDI and ethylenediamine core PAMAM generation 3.0 dendrimer cations generated using electrospray ionization (ESI) were sequentially isolated and mutually stored in a hexapole collision cell inside the mass spectrometer for a 2-second reaction period. The main product ion, the phosphopeptide dication, was isolated in the ICR cell and subjected to ECD. Sequence information obtained from this workflow was compared to CID analysis of phosphopeptide cations and anions.

This novel workflow (i.e., a charge inversion ion/ion reaction followed by ECD) is employed to provide full sequence information for phosphopeptides. This workflow is demonstrated using a reaction between a UOM9 phosphorylated PKC substrate-1 peptide (KRPpSQRHGSKY-NH2) anion [A-H]- and a multiply charged PAMAM [R+10H]10+ cation, which produces a doubly charged peptide cation [A+2H]2+. ECD fragmentation of [A+2H]2+ allows for elucidation of the full sequence and location of the phosphorylation. Conversely, CID of the [M+2H]2+ peptide cation results in dominant loss of the phosphate group and few b/y-type peptide fragment ions, leaving gaps in the peptide sequence and the location of phosphate group ambiguous. This sequential charge inversion ion/ion reaction and ECD workflow demonstrates improved sequence coverage and PTM localization of MALDI-generated phosphopeptide anions compared to traditional CID. Future work will entail applying this workflow to biological samples for sequencing key biomarkers of disease.

90. SPATIAL MAPPING OF SULFUR-CONTAINING METABOLITES IN A MOUSE MODEL OF SYSTEMIC STAPHYLOCOCCUS AUREUS INFECTION

Justin Ellenburg - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida; **Cristina Kraemer-Zimpel** - Michigan State University; **Paige Kies** - Michigan State University; **Neal D. Hammer** - Michigan State University; **Boone M. Prentice** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida

Introduction: During systemic infection, the prominent human pathogen Staphylococcus aureus will colonize numerous host organs, resulting in immune cell infiltrates that restrict further spread through the development of a macroscopic structure called an abscess. Abscesses are predicted to be nutrient-limiting environments. However, the spatial distribution of macronutrient sulfur across infected tissues represents a knowledge gap. In vitro studies have shown that S. aureus acquires organosulfur metabolites, such as glutathione, from the environment, but evidence of sulfur scavenging from host tissues is lacking. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) allows for the mapping and visualization of the spatial distribution of metabolites and provides insight into the complex chemical environment of staphylococcal abscesses.

Methods: Eight-week-old female C57BL/6 mice were infected with 107 CFUs of S. aureus strain JE2 via retro-orbital injection. Mice were euthanized 96 hours post-infection and the kidneys, livers, and hearts were harvested. Tissues were sectioned on a cryomicrotome (CM3050s, Leica Biosystems) at 10 µm thickness and thaw mounted onto indium tin oxide-coated glass slides. One section of S. aureus infected heart and one section of PBS-inoculated control heart were placed on each slide for comparison. Autofluorescence microscopy of slides was used to determine abscess localization. A 9-aminoacridine MALDI matrix layer was applied via sublimation using a custom-built apparatus and subsequently recrystallized. Tissues were analyzed in negative ion mode on a 7T solariX FT-ICR MS (Bruker Daltonics).

Results: In vitro testing identified ten sulfur-containing metabolites that S. aureus may be able to assimilate from exogenous sources. MALDI IMS analysis reveals three of these metabolites have disrupted distributions in infected tissues. Our data from mouse heart reveals discrete localization of cysteinyl glycine (CYS-GLY, m/z 177.034, 2.18 ppm) to the infectious foci (i.e., tissues abscess), as well as disruption of the normal relative abundances of glutathione (GSH, m/z 306.077, 0.88 ppm) and oxidized glutathione (GSSG, m/z 611.145, 0.42 ppm). Additional metabolites not identified with in vitro studies were also found to be disrupted during systemic infection. These include ascorbic acid (m/z 175.0251, 1.5ppm), cysteic acid (m/z 167.9975, 1.74ppm), and 3-sulfonatolactate (m/z 168.9814, 0.98ppm). The location of infectious foci has been confirmed via autofluorescence prior to IMS and via hematoxylin and eosin microscopy following IMS.

Conclusions: This work demonstrates a disruption to normal host redox activity and a potential for altered sulfur metabolism. Future work will focus on testing the statistical significance of replicate analyses.

91. TARGETING THE GUT MICROBIOME TO REDUCE THE BURDEN OF PROTEOTOXIC BACTERIA

Anan Safwat - 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; **Daniel Czyz** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

The human gut microbiota is a complex community affecting health and disease. Gut dysbiosis has been associated with age-dependent neurodegenerative protein conformational diseases (PCDs), including the most common Alzheimer's and Parkinson's diseases. Using Caenorhabditis elegans that express various tissue-specific sensors of protein folding, we screened all culturable isolates from the Human Microbiome Project for bacterial species that affect protein aggregation upon intestinal colonization. Among the detrimental proteotoxic bacteria,

we found Shigella spp. that consistently and significantly induced toxic protein aggregation in the host. Furthermore, recent studies revealed that Shigella reduces the abundance of butyrogenic bacteria, which are known to be protective against many ailments, including PCDs. While Shigella is most often associated with shigellosis, a gastro-intestinal infection, it can also colonize individuals asymptomatically. Their detrimental effect on the protective butyrogenic microbes, combined with the ability to disrupt protein folding and induce toxic aggregation, emphasizes the need for eradicating these microbes from the human gut. Antibiotics are used as a control method for targeting bacteria; however, they also disrupt the protective commensal microbiota and enrich for proteotoxic antibiotic-resistant strains. In the following study, we employed bacteriophages, viruses that specifically target and kill bacteria, to eliminate Shigella. We isolated three unique phages (Pepi, Seti, and Teti) against Shigella flexneri. Further characterization revealed their robust temperature and pH stability and exceptional specificity and efficacy in killing S. flexneri, making them ideal candidates for phage-mediated targeting of proteotoxic bacteria. Our approach can specifically eliminate detrimental bacteria, potentially restore gut eubiosis, and affect the pathogenesis of PCDs.

92. TOXIN AND CAPSULE PRODUCTION BY BACILLUS CEREUS BIOVAR ANTHRACIS INFLUENCE PATHOGENICITY IN MACROPHAGES AND ANIMAL MODELS

Treenate Jiranantasak - 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; **Donald J. Chabot** - Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University; **Arthur Friedlander** - Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University; **Christopher K. Cote** - Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University; **Richard Bowen** - United States Army Medical Research Institute of Infectious Disease; **Ian A. McMillan** - School of Life Sciences, College of Natural Sciences, University of Hawai'i at Mānoa; **Ted Hadfield** - Department of Geography, Emerging Pathogens Institute, 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; **Michael Norris** - Emerging Pathogens Institute, School of Life Sciences, College of Natural Sciences, University of Hawai'i at Mānoa

Bacillus cereus biovar anthracis (Bcbva) is a pathogen causing anthrax-like disease responsible for many wildlife mortalities in west and central Africa for decades. Genomic analyses revealed Bcbva as a member of the B. cereus species, even though it carries two plasmids, pBCXO1 and pBCXO2, which bear a striking resemblance to the pXO1 and pXO2 plasmids found in B. anthracis. The pathogenesis of anthrax is mainly due to the production of two secreted anthrax toxins and poly-γ-D-glutamic acid capsule (PDGA). Currently, the influence of Bcbva's sporulation, toxin, and capsule synthesis in infected macrophages and animals compared to B. anthracis Ames has not been well studied. Here, we demonstrated Bcbva was capable of multiplying, generating spores, and persisting in nutrient enrichment media for at least six days. Compared to

B. anthracis Ames, Bcbva sporulated faster but secreted significantly less protective antigen (PA). Bcbva secreted a significantly higher amount of attached PDGA capsule than B. anthracis Ames when cultivated in media containing normal human serum supplemented with CO2 mimicking natural host infection. Using in vitro studies, we discovered Bcbva spores could invade, germinate, and survive within mouse macrophages. Nevertheless, the internalization efficiency of Bcbva spores was much lower than B. anthracis spores. The virulence of Bcbva in vivo was further investigated by infecting Bcbva spores in Galleria mellonella larvae and guinea pigs. The median lethal doses (LD50) of Bcbva in G. mellonella larvae and guinea pigs were 20 and 1.5 times less than B. anthracis Ames, respectively. This suggests that Bcbva is more virulent than B. anthracis Ames. The pXO2 mutant Sterne B. anthracis strain has been used worldwide for vaccination among hoofstock. Several studies reported Sterne live spore anthrax vaccine induces host immune responses to toxin components and capsules of B. anthracis. Due to the high genetic similarity between the virulence plasmids of Bcbva and B. anthracis, we evaluated the effectiveness of Sterne vaccine against Bcbva infection in animals. The results indicated that vaccination of intranasally Bcbva-infected guinea pigs with Sterne vaccine causes 100% survival and no observable clinical signs in the animals implying protective immunity against disease. Further studies are needed to determine if a Sterne-based livestock program would be useful to reduce disease livestock across the known range of Bcbva, as it is known to affect livestock, hoofed wildlife, and several non-human primates; data are needed on the risks versus protective efficacy of Sterne vaccine in great apes.

93. UNVEILING THE ROLE OF REPRESSOR AND INTEGRASE GENES IN LYSOGENIC P2-LIKE PHAGES OF BURKHOLDERIA PSEUDOMALLEI

Pacharapong Khrongsee - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Iris irby** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Mariam Alami-Rose** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Jedsada Kaewrakmuk** - Faculty of medical technology, Prince of Songkla University, Hatyai, Songkhla, Thailand; **Apichai Tuanyok** - Department of Infectious Diseases and Pathology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Introduction: Burkholderia pseudomallei, the causative agent of melioidosis, presents significant challenges for both treatment and environmental decontamination in tropical regions. To address this, bacteriophages (phages) are under investigation as potential therapeutic and decontamination against this bacterium. Our research, employing diverse screening strategies, revealed that the broad host-range phages were indeed lysogenic phages present in specific strains of the bacteria. Despite the promise of lysogenic phages, their killing efficacy tests need improvement.

Method: We conducted a pan-genomic analysis on 135 complete genomes obtained from GenBank to identify hotspots of these phages in bacterial genomes. A comparative analysis of phage/prophage genomes revealed a correlation between site-specific recombination (SSR) sequences and the integrase gene within each prophage region. Transcriptomic profiling of tested prophages demonstrated high expression of the integrase and certain repressor genes during the lysogenic life cycle. Then, we performed mutagenesis on integrase and repressor genes using homologous recombination techniques in lysogens to test their functions.

Results: Remarkably, over fifty percent of bacterial genomes contained at least one prophage, providing a valuable resource for extracting

prophage sequences and isolating predicted temperate phages known to infect B. pseudomallei. Despite their prevalence, our killing efficacy tests of these phages yielded poor results. This prompted us to investigate how phage genes control their lytic-lysogenic life cycle. Surprisingly, the deletion of the integrase gene did not enhance the lytic switch. Instead, we observed effective improvement in the strictly lytic life cycle upon deleting the highly expressed repressor gene.

Conclusions: We concluded that the repressor gene plays a crucial role in the lytic lysogenic switch for these phages. This discovery provides a valuable clue for future application of bacteriophage engineering.

94. A SYSTEMATIC REVIEW AND META-ANALYSIS ON SWINE VIRAL PATHOGENS IN CHINA: TRENDS, PREVALENCE, AND IMPLICATIONS FOR PUBLIC HEALTH AND BIOSECURITY

Thanaporn Thongthum - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Kaiyu Zhou** - Department of Biology, Emory University; **Benjamin D. Anderson** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: China is the world's largest producer of pork, accounting for a substantial portion of global production. Despite the critical implications of economic productivity and potential impact on public health due to outbreaks, there is a lack of a comprehensive national-scale review on swine pathogens in China. This study aims to analyze publication trends related to swine pathogens, presuming that these trends may indicate the circulation of swine pathogens in the country, with a primary focus on characterizing their temporal and geographic patterns.

Methods: Following PRISMA guidelines, the study reviewed 7,268 articles across PubMed, Web of Science, and CAB Abstracts databases, ultimately incorporating 691 articles for data extraction. After eligibility assessment, 418 studies focusing on viral pathogens were included for extraction. Extracted data covered location, sampling site, farm size, pathogen name and strain, production stage, health status, sample collection time, diagnostic method, reported prevalence, and total sample number. Following data quality assessment, 340 studies were included for quantitative analysis. Statistical analysis calculations and the generation of forest plots were conducted using SAS version 9.4. ArcGIS Pro was utilized for visual mapping.

Results: Pseudorabies virus (PRV), porcine circovirus type 2 virus (PCV2), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory virus (PRRSV) emerged as the most studied pathogens, with forest plots suggesting that prevalence stabilized over time. However, the pooled prevalence of these pathogens was relatively high: 40.7% for PRV, 50.4% for PCV2, 53.4% for PEDV, and 39.1% for PRRSV. These pathogens were consistently studied over the last decade across provinces in China. The geographic density of studies showed a high concentration in the eastern and southern provinces, especially Shandong, Henan, Jiangsu, and Guangdong. Due to inconsistency in reporting and considerable between-study heterogeneity, statistical tests found no significant associations between prevalence and production stages, pig health status, or farm size.

Implications: The findings reveal a concerning and persistent prevalence of major swine pathogens, including PRV, PCV2, PEDV, and PRRSV in Chinese pig populations. The stability of prevalences of studied pathogens over time suggests an ongoing threat to animal and public health, necessitating continuous monitoring and stringent biosecurity measures. The lack of significant associations between prevalence and production stages, pig health status, or farm size due to reporting inconsistencies and considerable study heterogeneity underscores the need for standardized and comprehensive research.

95. EMERGING VIRAL PATHOGENS IN FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA

An-Chi Cheng - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Pedro H. de Oliveira Viadanna** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Laura Roldan** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Kristen Wilson** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Tracey D. Logan** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **John Lednicky** - Department of Infectious Disease, 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

White-tailed deer (Odocoileus virginianus, WTD) is one of the most widely distributed wildlife species in the United States and deer farming is a growing industry in Florida. Viral hemorrhagic diseases and bacterial infections have been identified as significant contributors to mortality in farmed WTD in Florida, resulting in substantial production loss and potential threats to wildlife. Nevertheless, a knowledge gap remains regarding other emerging viral pathogens in Florida's WTD population that could similarly impact production. This study aims to elucidate the special heterogeneity, possible origins, and transmission routes for bovine viral diarrhea virus (BVDV) and mule deerpox virus (MDPV), both identified as emerging viral pathogens in the Florida WTD. The University of Florida Cervidae Health Research Initiative (CHeRI) provides diagnostic services to Florida deer farmers for the determination of cause of death and disease surveillance. From 2016 to date, participating Florida ranches provided recently deceased farmed WTD or shipped samples for necropsy

and analysis through the CHeRI diagnostic program. We identified four cases of BVDV with severe hemorrhage on two farms in Florida WTD in 2018. The genome sequence data showed that our BVDV are highly similar to the BVDV2a found in beef cattle in other states in the US, suggesting the possibility of cross-species transmission. This is the first report of BVDV in Florida WTD; with this finding, disease surveillance is crucial at the wildlife-livestock interface to prevent potential outbreaks among host species. Since 2017, a comprehensive necropsy analysis of 148 cases exhibiting skin lesions has yielded significant findings, with 50 cases testing positive for MDPV. MDPV cases exhibit a statewide distribution in Florida, particularly impacting animals aged 2 to 12 weeks, with the highest case numbers occurring during the summer months. Our results show that MDPV can be detected through conventional PCR from not only skin and tongue lesion tissue and swabs, but also from most internal organ tissue. Genome data showed that the MDPV in this study is identical to the first detected MDPV in Florida in 2016, suggesting that MDPV is conservative over time within the farmed deer population. Future works aim to understand viral ecology by identifying possible vectors and incorporating animal transmission data. Our results will provide valuable information to improve preventative health measures of both wild and captive WTD in Florida, especially at the wildlife-livestock interface.

96. HAZARDOUS DRINKING ONSET, PROGRESSION, AND CONTINUATION AMONG WOMEN WITH HIV: A QUALITATIVE ASSESSMENT OF LIFETIME DRINKING HISTORY

Christina Parisi - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Abigail Gracy** - Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Sashaun Ranger** - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Rebecca Fisk-Hoffman** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Nanyangwe Siuluta** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Isaac Payton** - Emerging Pathogens Institute, College of Nursing, University of Florida; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Shantrel Canidate** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: Hazardous alcohol use is common among people with HIV (PWH), and women with HIV (WWH) with hazardous drinking patterns disproportionately experience poor HIV outcomes compared with men with HIV. The aim of this analysis was to identify important factors in the onset, progression, and continuation of hazardous drinking patterns among a sample of WWH enrolled in a clinical trial to reduce their drinking to better inform future treatment and intervention.

Methods: Twenty WWH with a history of hazardous drinking patterns (>7 drinks/week) were recruited from participants who had completed the WHAT-IF? Study, a clinical alcohol intervention using naltrexone designed to reduce hazardous drinking among WWH. Participants completed in-depth, semi-structured interviews. Interview questions included reasons participants began drinking, lifetime drinking history, and current drinking patterns post-study. All transcripts were transcribed verbatim and were assessed using thematic analysis.

Results: Among twenty WWH (mean age 49.3, 85% Black, 5% Hispanic), reasons to begin drinking included social factors such as “peer pressure” or normalization of drinking by family/friends. WWH in the study often discussed the effect of the age of drinking onset (e.g., preadolescent, adolescent, young adult) on their subsequent drinking patterns. Participants also frequently differentiated between when they tried their first drink and when they began drinking regularly and noted the importance of this distinction. Progression to hazardous drinking patterns was characterized by enjoying drinking, on and off patterns of drinking, co-use with other substances, substituting alcohol for drugs, and using alcohol to cope with trauma or emotional issues like depression and anxiety. Many of those factors were reasons why WWH continued to drink in the present. Additional reasons included perceived health benefits from their continued alcohol use, such as improved sleep.

Conclusions: There were many factors identified by WWH that influenced their lifetime drinking onset, progression, and present drinking. Considering the wide variety of factors that influenced alcohol use, context-specific primary, secondary, and tertiary interventions may be required at different timepoints in the lifetime of PWH to prevent hazardous alcohol use that affects overall and HIV-related health outcomes.

97. INTEGRATION OF A RNA EXTRACTION DEVICE WITH A PORTABLE REAL-TIME DETECTOR FOR HIV TESTING AT POINT-OF-CARE

George Adedokun - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida; **Gurjit Sidhu** - Department of Medicine, College of Medicine, University of Florida; **Gary Wang** - Department of Medicine, College of Medicine, University of Florida; **Z. Hugh Fan** - Department of Medicine, College of Medicine, University of Florida

Human immunodeficiency virus (HIV) has killed over 40.4 million lives. Effective HIV detection during the seroconversion period can help reduce mortality. However, existing molecular testing methods are complex, time-consuming, and expensive, and therefore not suitable for use in resource-limited settings. Low-cost, point-of-care (POC) diagnostics devices with molecular testing will address the challenge. To meet this need, we have developed a miniaturized device for the detection of HIV that uses cellulose paper as a nucleic-acid-extraction substrate, followed by isothermal amplification and detection.

The RNA extraction device is 3-D printed, and it is integrated with a real-time detector for quantitative virus load. The device comprises three parts: (1) a buffer unit with 4 wells for lysis, binding, and two wash buffers, (2) a mixing unit, and (3) a detection unit. The mixing unit slides against the buffer unit, actuating a mini-valve in each well for sequential delivery of the solutions. The concept of the ball-based valve is borrowed from a ballpoint pen, in which ink is dispensed onto paper when the metal ball at the tip is pressed while writing. The detection unit is an assembly of a polycarbonate well layer and a laminated chromatography paper, and it is attached to the bottom of the mixing unit. The operational steps, including sample introduction, the release of the reagents for lysis/binding/washing, and the enrichment and purification of nucleic acid on the paper in the detection unit.

The collected RNA is then amplified by (1) moving the detection unit into a real-time detector, which is made of a digital microscope mounted on a 3D-printed stand, an integrated circuit, heater, and battery (2) reverse

transcription loop-mediated isothermal amplification (RT-LAMP). The microscope aligns with the detection unit above the heater to collect RT-LAMP signals. Captured images are analyzed using Python codes, generating a standard curve of fluorescence intensity over time. Our real-time detector provides quantitative virus load information and faster assay times for positives.

The RT-LAMP assay exhibited a remarkable limit of detection, detecting as few as 4 copies/μL of HIV in human serum, with no cross-reactivity observed with hepatitis C virus (HCV). Clinical validation using samples from potential HIV-infected patients with confirmed HCV co-infection demonstrated 100% agreement with results from a commercial PCR instrument, ensuring 100% specificity.

Our miniaturized device provides critical functions for POC HIV detection: sample preparation and detection. It is low-cost, battery-powered, highly sensitive, and specific, with potential for HIV diagnosis in resource-limited settings.

98. MUSEOMICS AND META-TRANSCRIPTOMICS FOR VIRAL DISCOVERY AND MICROBIOME CHARACTERIZATION OF FRESH BAT GUANO SAMPLES, FROZEN AND FLUID-PRESERVED BAT TISSUES

Maclean Bassett - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, College of Medicine, University of Florida; **Jakob Beardsley** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, University of Florida; **Massimiliano Tagliamonte** - Interdisciplinary Center for Biotechnology Research, University of Florida; **Lisa Smith** - Florida Fish and Wildlife Conservation Commission; **Marco Salemi** - Department of Pathology, Immunology, and Laboratory Medicine, Emerging Pathogens Institute, University of Florida; **Eddie Holmes** - University of Sydney; **Simone Marini** - Department of Pathology, Immunology, and Laboratory Medicine, University of Florida; **Elizabeth Braun de Torrez** - Florida Fish and Wildlife Conservation Commission; **Verity Mathis** - Florida Museum of Natural History; **Carla Mavian** - Department of Pathology, Immunology,

and Laboratory Medicine, Emerging Pathogens Institute, University of Florida

Introduction: Zoonotic pathogens discovered by investigations of the bat microbiome (bacteriome and virome) may shed light on the diet and disease state of their hosts as well as their potential for zoonosis. The Florida Natural History Museum maintains a collection of fluid-preserved whole bat specimens which could serve as valuable sources of genetic information for insight into the history of Florida's bat populations and ecologically connected species. Additionally, collection and analysis of fresh guano samples from bat roosts serves as a useful comparison with historic samples for monitoring trends in microbiome composition change over time and for the identification of currently circulating pathogens in Florida bat populations.

Methods: Rectum tissue was resected from frozen-, ethanol-, and formalin- preserved museum samples from Florida insectivorous bats. Tadarida brasiliensis bats were captured near Camp Blanding in Florida, and anal swabs and fecal samples were collected. Tissues, swabs, and guano underwent DNA and RNA extractions. cDNA was generated into shotgun libraries (metagenomic and meta-transcriptomic) or 16S amplicon libraries (microbiome profiling) and sequenced using Illumina NovaSeq or MiSeq. Reads were analyzed using QIIME2, Kraken2, and MetaPhlAn4 to profile the bacterial microbiome composition. Antibiotic resistance genes were profiled using KARGA. Contigs were assembled using MEGAHIT and input to ncbi blastn for viral discovery. Maximum likelihood trees were generated using IQ-TREE.

Results: Multiple microbial resistance genes, including multi-drug and multi-metal resistance, were found with high support in the fresh samples suggesting the presence of microbial populations with diverse antimicrobial resistances in the gut microbiota of Florida bat populations. Viral metagenomic discovery analysis suggested the presence of multiple novel viruses. The full capsid sequence of an astrovirus and the spike of an alphacoronavirus were notably identified. Maximum likelihood trees support the presence of the novel viruses based on the clustering of the contigs with related series.

Conclusions: The results of this study provide an important platform for future work with frozen and fluid-preserved bat gut tissue or fresh bat guano and their use in viral discovery investigations as well as providing further insight into the bat microbiota via metagenomic profiling. This work may aid in furthering the understanding of viral tolerance and circulation in Florida bat populations which could play a role in new cycles of zoonotic transmission.

99. ONE-POT RT-LAMP CRISPR/CAS12B PLATFORM FOR RAPID DETECTION OF TILAPIA LAKE VIRUS

Dorothea Megarani - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida; **Lilia Yang** - Department of Chemical Engineering, College of Engineering, University of Florida; **Eva Marie Quijano Cardé** - Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis; **Esteban Soto** - Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis; **Roy Yanong** - Institute of Food and Agricultural Sciences, University of Florida; **Kuttichantran Subramaniam** - Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

Tilapia lake virus disease (TiLVD) is a viral disease that has been associated with high morbidity and mortality in cultured and wild tilapia worldwide. Although several diagnostic tools have been developed for TiLV detection, they require advanced equipment and time-consuming procedures, making them impractical for laboratories with limited resources or pondside use. Addressing this challenge, we have developed and partially validated an innovative, rapid, and cost-effective one-pot diagnostic assay that combines thermostable Cas12b enzyme with RT-LAMP amplification, targeting a conserved region within segment 4 of the genome. Notably, this assay can be conveniently used, as the incubation process is done at 62°C for 75 minutes, and the results can be observed using an inexpensive portable fluorescence viewer. The TiLV one-pot assay is sensitive and specific, with a limit of detection of 50 RNA viral copies, and no cross-reaction was detected with other fish DNA and RNA viruses. In addition, the assay could also detect 12 TiLV transcripts from

other regions despite primer mismatches. The analysis of 98 positive and 94 negative samples previously determined by TaqMan qPCR assay resulted in both diagnostic sensitivity and specificity of 96.9% and 100%, respectively. Additional samples from different populations or field outbreaks will be utilized to validate the diagnostic performance of the assay further. The present study outlines a report on the development and partial validation of a diagnostic assay for TiLV in stages 1 and 2, following the guidelines from WOAH. Finally, this assay can serve as a valuable tool in global surveillance efforts that fish health professionals can easily employ to screen, monitor, and control TiLV disease in tilapia production.

100. TIME SINCE HIV DIAGNOSIS IS ASSOCIATED WITH MEMORY IMPAIRMENT

Mark K. Britton - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Jason DeFelice** - Department of Clinical and Health Psychology, 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; **Gladys Ibanez** - Department of Epidemiology, Department of Epidemiology, Robert Stempel College of Public Health and Social Work, Florida International University; **Charurut Somboonwit** - Department of Internal Medicine, College of Medicine, University of South Florida; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Joseph Gullett** - Department of Clinical and Health Psychology, 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

Introduction: As people with HIV (PWH) age, they experience age-related comorbidities earlier and more frequently than people without HIV (PWoH). These comorbidities may include neurodegenerative changes, and emerging evidence suggests that older PWH are more likely than PWoH to exhibit amnestic mild cognitive impairment (aMCI), or abnormal

memory decline that impedes daily functioning. Given that little is known about the drivers of this association, we tested associations between aMCI, non-amnestic MCI (naMCI), and putative clinicodemographic risk factors.

Methods: 56 older adults with HIV (age range: 59-77, mean = 64.3, SD = 4.43; 57% female; 64% Black) completed a neurocognitive battery assessing cognitive domains impaired in HIV, aMCI, or both. Impairment in daily activities was assessed with the self- and informant-reported Functional Activities Questionnaire (FAQ). Consensus diagnoses (aMCI/naMCI/unimpaired) were assigned by two neuropsychologists (JG and RAC) based on the available cognitive and FAQ data. Whole blood was collected, and the APOE4 genotype (carrier/noncarrier) was assessed. Lifetime exposure to cocaine, alcohol, and opioids was quantified using the Kreek-McHugh-Schluger-Kellogg scale (KMSK). Age, years since HIV diagnosis, years of education, and antiretroviral therapy adherence (≥ 95%/<95%) were assessed by self-report. Associations between MCI status and HIV diagnosis were assessed with chi-squared tests (categorical predictors) and Kruskal-Wallis tests (continuous predictors).

Results: MCI status was associated with years since HIV diagnosis, with a moderate effect size (H[(2]) = 8.26, p = 0.016, η2 = 0.19); post-hoc Dunn tests revealed that aMCI was associated with a longer time since diagnosis relative to both naMCI (27.19(6.16) vs. 20.15(9.05) years, padj = 0.034) and unimpaired groups (27.19(6.16) vs. 19.33(8.13) years, padj = 0.028). Time since diagnosis was uncorrelated with age (r = 0.17, p = 0.23). MCI status was also associated with lifetime opioid exposure, with a medium effect size (H(2) = 7.82, p = 0.020, η2 = 0.11); post-hoc tests revealed greater opioid exposure among the aMCI group than the unimpaired group (z = -2.73, padj = 0.019). MCI status was not significantly associated with age, APOE4 genotype, years of education, ART adherence, cocaine exposure, or alcohol exposure (ps > 0.05).

Conclusions: Time since HIV diagnosis and opioid exposure are associated with aMCI; these associations are not driven by chronological age. These findings may reflect longer exposure to low-level inflammation or long-

term effects of untreated HIV prior to widespread ART availability and/or the behavioral or physiological correlates of opioid use.

101. TORQUE TENO SUS VIRUS 1: A POTENTIAL SURROGATE PATHOGEN TO STUDY PIG-TRANSMITTED TRANSBOUNDARY ANIMAL DISEASES

Xiaolong Li - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Brandon Parker** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Raoul K. Boughton** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, 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; **Timothy J. Smyser** - National Wildlife Research Center, United States Department of Agriculture; **James D. Austin** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Kim M. Pepin** - National Wildlife Research Center, United States Department of Agriculture; **Ryan S. Miller** - Center for Epidemiology and Animal Health, United States Department of Agriculture; **Kurt C. Vercauteren** - National Wildlife Research Center, United States Department of Agriculture; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Understanding the epidemiology and transmission dynamics of transboundary animal diseases (TADs) among wild pigs (Sus scrofa) will aid in preventing the introduction or containment of TADs among wild populations. Given challenges associated with studying TADs in free-ranging populations, a surrogate pathogen system for in situ studies of pathogen dynamics would be ideal to better predict how pathogens may circulate and be maintained within populations, how they may spillover into domestic populations, and how disease spread may be mitigated. We assessed the suitability of Torque teno sus virus 1 (TTSuV1) to serve as a surrogate pathogen for molecular epidemiological studies in wild pigs by

investigating the prevalence, virulence, persistence, and genetic variability at two study areas: Archbold's Buck Island Ranch in Florida and Savanna River Site in South Carolina. We then conducted a molecular epidemiological case study within the Archbold's Buck Island Ranch site to determine how analysis of this pathogen could inform transmission studies. Prevalence was high at both study areas (40%, n=190). Phylogenetic analyses revealed high levels of genetic variability within and between study sites. Our case study showed that pairwise host relatedness and geographic distance were highly correlated to pairwise viral genetic similarity. Molecular epidemiological analyses revealed a distinct pattern of direct transmission from pig to pig occurring within family groups. Our results suggest TTSuV1 is highly suitable for molecular epidemiological analyses and will be useful for future in situ studies of transmission dynamics in wild pigs.

102. TRAUMA AND SELF-REPORTED CERVICAL CANCER SCREENING AMONG WOMEN LIVING WITH HIV: RESULTS FROM THE FLORIDA COHORT STUDY

Nanyangwe Siuluta - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Caroline Deaterly** - Department of Medicine, University of Alabama at Birmingham; **Karina Villalba** - University of Central Florida; **Nichole M. Scaglione** - Department of Health Education and Behavior, University of Florida; **Yancheng Li** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Jacqueline Sherbuk** - Department of Infectious Disease, University of South Florida; **Maya Widmeyer** - Comprehensive Health Care; **Robert Cook** - Department of Epidemiology, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida

Introduction: Women with HIV (WWH) are more likely than the general population to experience trauma. Trauma in WWH is associated with delayed cervical cancer screening, yet the specific associated traumatic experiences or time periods causing the delay in cervical cancer screening remain unknown. This study aimed to establish the association between

specific traumatic experiences (lifetime and before 18 years of age) and self-reported cervical cancer screening among WWH.

Method: A secondary data analysis of 91 WWH from clinics in Florida (2019-2022) was conducted. These participants were 50+ years old (62%), Black (53%), White (32%), and Hispanic (15%). Participants were asked to report 12 traumatic experiences (i.e., sexual harassment, unwanted sexual touch, forced sex, transactional sex, discrimination, physical attack, being stalked, hate crimes, verbal, physical, emotional, and sexual abuse) across different timings of exposure (i.e., lifetime, before 18 years of age and in the past 12 months). Participants were also asked to report their cervical cancer screening history, specifically if they last received a cervical pap smear or HPV test from a healthcare provider within three years. SAS V9.4 software was used for Chi-square, Fischer's exact test, and Logistic regression analysis.

Results: The prevalence of specific traumatic events among this population varied from 1% and 64%. Sexual harassment was reported throughout their lifetime (26%) and before 18 years of age (15%). Notably, 20% reported not receiving cervical cancer screening within three years. WWH who experienced sexual harassment before 18 years of age were statistically significantly less likely to receive cervical cancer screening within three years compared to those who had not experienced sexual harassment before 18 years of age (42% vs 16%, OR=3.7, 95%CI 1.0-14).

Conclusions: WWH who had experienced sexual harassment before 18 years of age, were at high risk for not receiving cervical cancer screening from a health care provider within three years. Therefore, more innovative alternative methods such as self-sampling for cervical cancer may need to be explored for this vulnerable population.

103. A GLOBAL ANALYSIS OF BACTERIAL GENOMES LINKS SECONDARY METABOLISM AND ANTIBIOTIC RESISTANCE

Murrel Saldanha - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **José Cediel-Becerra** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Shannon Manning** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Francisco Barona-Gómez** - Institute of Biology Leiden, Leiden University, Leiden, South Holland, Netherland; **Marc Chevrette** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

A rapid rise in resistant pathogenic infections coupled with the decline in the development of new antibiotics makes the immediate discovery of novel antimicrobials of critical importance. Bacteria are prolific producers of secondary metabolites, many of which contributed to the 'Golden Age' of antibiotic discovery in the mid 20th century. There has been a resurgence in interest in identifying and isolating novel secondary metabolites synthesized by bacterial biosynthetic gene clusters (BGCs), given their known therapeutic potential. Although BGCs producing antimicrobial compounds may also house corresponding resistance genes, a quantitative link between BGCs and resistance remains unclear. To tackle this problem, we have developed a comparative genomic pipeline to mine bacterial genomes for BGC families and resistance genes in 10 well-studied taxonomic groups (including Mycobacterianales, Pseudomonales, Kitastatosporales, etc.). Genomes for all groups were retrieved from NCBI Genbank database and Natural Products Discovery Center and subsequently mined for BGCs using antiSMASH. Given the size of the database BiG-SLiCE was used to cluster BGCs. CARD's (Comprehensive Antibiotic Resistance Database) Resistance Gene Indentfier software and Resfams were used to identify genome specific resistomes. Leveraging bioinformatic tools we have investigated the

correlation between BGCs and AMR through an evolutionary lens. This large-scale genomic analysis aims to – (1) Highlight new bacterial groups/species/BGCs of interest for drug discovery studies based on their AMR profile, (2) Profile AMR genes for clinically relevant pathogens globally vs located in BGCs specifically, (3) Reconstruct phylogenies to gain insights into cross-species distribution of biosynthetic gene cluster families and estimate degree of lateral inheritance of resistance elements.

104. ASSESSING VACCINE EFFECTIVENESS IN TEST-NEGATIVE DESIGN DATA BY RECURRENT-EVENT COX REGRESSION

Shangchen Song - Department of Biostatistics, College of Public Health and Health Professions, University of Florida; **Ira Longini** - Department of Biostatistics, College of Public Health and Health Professions, University of Florida

The widespread availability of COVID-19 testing has enabled the use of test-negative designs (TNDs) for evaluating vaccine effectiveness (VE) in relation to the disease. These designs involve enlisting individuals who display COVID-19 symptoms and seek medical attention, subsequently testing them for the virus to distinguish cases from controls. TND helps reduce selection bias stemming from healthcare-seeking behaviors. Nonetheless, the prevalent method for analyzing TND data is restricted to logistic regression, which may be inadequate when temporal confounding occurs. In this study, we proposed an innovative rationale to regard the TND as a special cohort study which estimates a critical metric for burden on the medical system. Then, a recurrent-event Cox regression model with time-dependent covariates was proposed to account for infection-specific VE as well as time-varying vaccination. Finally, we presented the results of the methods applied to both simulated and real-world TND data.

105. ASSESSMENT OF ANTIBIOTIC RESISTANCE GENES (ARGS) IN FISH SAMPLES FROM GREAT LAKES, MICHIGAN, UNITED STATES OF AMERICA

Benjamin D. Anderson - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Thanaporn Thongthum** - Department of Environmental and Global Health, Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Alvin Wu** - Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Joanne Wang** - Emerging Pathogens Institute, College of Public Health and Health Professions, University of Florida; **Joseph H. Bisesi** - Department of Environmental and Global Health, Center for Environmental and Human Toxicology, College of Public Health and Health Professions, University of Florida; **Bridget B. Baker** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Public Health and Health Professions, University of Florida; **Tracie R. Baker** - Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida

Background: Antibiotic resistances are a global public health concern, with environmental reservoirs serving as potential hotspots for the emergence and dissemination of antibiotic resistance genes (ARGs). The Great Lakes, vital freshwater ecosystems and a primary drinking water source, face potential antibiotic contamination, particularly with the common detection of sulfamethoxazole in surface water along the Lake Huron to Erie corridor. Fish in the Great Lakes, especially wild-caught Walleye (WAE) and Yellow Perch (YEP), can act as bioindicators of environmental contamination. Given their popularity in diet, these fish also pose a potential source of ARG exposure for people. Understanding ARG prevalence in Great Lakes fish and identifying associated risk factors is crucial for assessing environmental and public health risks linked to antibiotic pollution in the Great Lakes.

Methods: A total of 20 fish were sampled from the Rouge River and Belle Isle (10 each). From each sampling site, there were 5 WAE and 5 YEP samples. Five different organs (gill, intestine, live, muscle, and stomach)

were dissected from each fish, giving a total of 100 samples. DNA from each sample was extracted for the molecular detection of ARGs through the quantitative chain reaction (qPCR) with 21 targeted ARGs. The qPCR was conducted on Quant Studio 3, and Ct values under 38 were considered positive. All statistical analyses were performed using SAS version 9.4.

Findings: The tetW gene was the most prevalent ARG (78%), followed by sulI (62%) and sulIII (61%). Other noteworthy ARGs included tetG (38%), ampr (36%), and ermB (25%), associated with tetracycline, β-lactam, and macrolide resistance, respectively. On the other hand, tetO, blaCTX_M, and blaTEM were not detected in any samples. While no significant associations with sampling sites were found, fish species and organs emerged as significant risk factors for ARG uptake. Odds of uptake for any ARGs were 2.52 times higher in WAE than in YEP and 1.75 times lower in the liver than in the stomach. Overall, fish species is a key risk factor of the uptake of tetW, sul(I), ampr, ermB, tetS, and ereA.

Implications: Although differences between study sites were not statistically significant, a higher likelihood of ARG uptake of WAE than YEP suggests potential impacts of trophic levels and spatial ranges on ARG uptake. The notable ARG prevalence in muscles and stomach highlights the importance of understanding potential antibiotic resistance transmission from fish to humans, given the significance of muscle consumption in human diets.

106. AUTOMATIC CANCER CELL TAXONOMY USING AN ENSEMBLE OF DEEP NEURAL NETWORKS

Se-woon Choe - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Jin-woo Jeong** - Department of Biomedical Engineering, Seoul National University of Science and Technology; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Microscopic image-based analysis has been intensively performed for pathological studies and diagnosis of diseases. However, mis-authentication of cell lines due to misjudgments by pathologists has been recognized as a serious problem. To address this problem, we propose a deep-learning-based approach for the automatic taxonomy of cancer cell types. A total of 889 bright-field microscopic images of four cancer cell lines were acquired using a benchtop microscope. Individual cells were further segmented and augmented to increase the image dataset. Afterward, deep transfer learning was adopted to accelerate the classification of cancer types. Experiments revealed that the deep-learning-based methods outperformed traditional machine-learning-based methods. Moreover, the Wilcoxon signed-rank test showed that deep ensemble approaches outperformed individual deep-learning-based models ($p < 0.001$) and were in effect to achieve the classification accuracy up to 97.735%. Additional investigation with the Wilcoxon signed-rank test was conducted to consider various network design choices, such as the type of optimizer, type of learning rate scheduler, degree of fine-tuning, and use of data augmentation. Finally, it was found that the using data augmentation and updating all the weights of a network during fine-tuning improve the overall performance of individual convolutional neural network models.

107. CHARACTERIZING THE MODE OF ACTION OF A NATURAL INSECTICIDE ISOLATED FROM PATCHOULI OIL

Zhilin Li - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Rui Chen** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Sarah E. McComic** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Steve Bessette** - Pyrone Systems Inc. San Diego, California, USA.; **Edmund Norris** - USDA/ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608, USA.; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Jeffery R. Bloomquist** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

The development of insecticide resistance highlights the demand for novel chemicals for pest control. Natural products have been and continue to be a key source for insecticide development. Compound A, a natural product isolated from patchouli oil, was found to be highly toxic to mosquitoes and cockroaches with 24-hr toxicity LD50 at 96.2 ng/mg and 33.7 ng/mg respectively, and knockdown potency equal to DDT, with 1-hr KD50 at 11.1 ng/mg, suggesting compound A is a promising insecticide and has a neural mode of action. For this study, our goal was to determine the mechanism of toxicity for this natural compound. To test if compound A is a neurotoxin, We first conduct recordings on the Drosophila larval central nervous system. Compound A was found to significantly reduce the electrical activity at 10 μM. Additionally, we found this compound induced dose-dependent excitation in cockroach peripheral sensory nerves and the excitation spikes resemble that of DDT. Dose-dependent effects on both central and peripheral nerves suggest this compound can cause poisoning globally in the nervous system. To examine if this compound affects the insect muscle system, we employed the neuromuscular conjunction recording technique where our data

showed that this compound can induce excitatory postsynaptic potential (EPSP) bursts without nerve stimuli from house fly larval muscle, which suggests the target is on the presynaptic nerves. Depolarization on mosquito larval muscle membrane indicated the target protein is also present in the muscle cell. Muscle Contractions were prevented and reversed by tetrodotoxin which is a specific blocker of voltage-gated sodium channels, suggesting this compound is an agonist of voltage-gated sodium channels. This speculation was supported by our experiment with cockroach giant axons where action potential bursts and depolarizations were induced by this compound at 30 μM without external stimuli. To test if voltage-gated potassium channels (Kv) were another target of this compound, we did contact toxicity bioassays on three Kv Drosophila mutants, among which the Shab Kv2 mutant strain showed 1.8-fold resistance to the compound when compared to the wild strain. We also found that compound A blocked the current with patch-clamped HEK293 cells expressing Anopheles gambiae Kv2 channels, which suggested this compound is an antagonist of Kv channels. Taken together, our data suggested compound A is both an agonist of the voltage-gated sodium channels and an antagonist of the voltage-gated potassium channels. We are currently working on voltage-clamp experiments on cockroach DUM neurons to examine its direct effect on voltage-gated sodium channels.

108. CHASING WAVES: SEAWATER-INDUCED AND COLD SHOCK EFFECTS IN ANTIBACTERIAL SECONDARY METABOLITES IN MARINE STREPTOMYCES

Andres Cumsille - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida; **Andres M. Caraballo-Rodriguez** - Skaggs School of pharmacy and pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA.; **Bastien L'Hermitte** - Institut de Génétique et de Développement de Rennes (IGDR), Univ. Rennes, Rennes, France.; **Nestor Serna-Cardona** - Centro de Biotecnología DAL, Universidad Técnica Federico Santa María, Valparaíso, Chile; **Reynald Gillet** - Institut de Génétique et de Développement de Rennes (IGDR), Univ. Rennes, Rennes, France.; **Pieter C. Dorrestein** - Skaggs School of pharmacy and pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA.; **Marc Chevrette** - Department of Microbiology and Cell Science, Emerging Pathogens Institute, University of Florida; **Beatriz Camara** - Centro de Biotecnología DAL, Universidad Técnica Federico Santa María, Valparaíso, Chile

Introduction: Antimicrobial resistance represents a significant global health threat. ESKAPE pathogens, notorious for causing hard-to-treat infections, are a key area of concern. The development of novel antibiotics to treat emerging pathogens is heavily reliant on small molecules, also known as natural products, especially those produced by the bacterial genus Streptomyces; over half of known antimicrobials are derived from bacterial natural products. Traditionally, discovery efforts have focused on soil Streptomyces, while marine ecosystems, which may harbor new biosynthetic pathways for novel antibiotics, remain underexplored. Natural product biosynthesis in bacteria is complex, and many strains do not fully express their biosynthetic potential under standard laboratory conditions. To address this, varying cultivation conditions can enhance the production of a wider range of molecules.

Methods: In this study, five Streptomyces strains from diverse marine ecosystems in Chile were examined to induce antibacterial metabolites. Three conditions were tested: growth time, Artificial Sea Water (ASW) media, and cold shock (5°C). Methanol crude extracts were tested for

antibacterial activity against ESKAPE pathogens. Furthermore, a dereplication step, crucial for identifying and excluding known compounds, was conducted using liquid chromatography tandem mass spectrometry (LC-MS/MS)-based metabolomics and molecular network analysis. Additionally, the genomes of these strains were sequenced to analyze their biosynthetic gene clusters (BGCs).

Results: The presence of ASW significantly influenced the metabolomic profiles of the strains, more than growth time, with the cold shock showing minimal impact. Two strains displayed antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA). In particular, Streptomyces sp. H-KF8 produced distinct chemical profile with antibiotic activity in the presence of ASW after seven days of culture. H-KF8 strain yielded a diverse range of metabolites exclusive to ASW conditions. In silico analyses suggested some of these as 8-11 amino acids peptidic products. The genome of H-KF8 revealed a non-ribosomal peptide synthetase BGC potentially responsible for this molecular family. Further experiments are planned to fully characterize and confirm their chemical structure to correctly link the BGCs involved in their biosynthesis.

Conclusions: This study highlights the potential of mimicking natural environmental conditions to discover diverse antibacterial compounds, showing promise against MRSA. It underscores marine environments as significant sources for antibiotic discovery.

109. CLIMATE EXTREMES AND SOCIAL VULNERABILITY IN THE ALABAMA BLACK BELT

Julia Tootle - Department of Biomedical Engineering, College of Engineering, University of Florida; **Sadie Ryan** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Introduction: The Alabama Black Belt (ABB) is a region of the United States that has been historically susceptible to extreme poverty and climate-change-driven social and racial-based, environmental injustice. Comprised of 19 counties extending from Alabama's western border with Mississippi to its near eastern border with Georgia, the ABB was recently established as the Alabama Black Belt National Heritage Area. With this new designation and its underserved history, there is a need for current investigations regarding future climate-change-driven challenges and their connection with the region's social vulnerability.

Methods: The Climate Extreme Index (CEI) is a method utilized to capture the prevalence of climate extremes in a defined geographical area and create a framework for analysis. Recent research efforts have modified the CEI, in which four climate indicators [Maximum Temperature (Tmax), Minimum Temperature (Tmin), Soil Moisture (SM), and Precipitation (P)] were used to develop a Z-score-based CEI analysis. To calculate the CEI, the annual Z-score (1850 to 2100) is calculated for each of the four climate vectors (Tmax, Tmin, P, SM) for a given CMIP6 future climate model, Shared Socioeconomic Pathway scenario, and month. Applying Chebyshev's Rule, we examine each of the four Z-score vectors and determine what threshold is appropriate to establish an annual Z-score as extreme, resulting in each indicator receiving a re-scored value of 0 (non-extreme) or 1 (extreme). The CEI is then calculated for each year by summing the values assigned to each of the four parameters (ranging from 0 to 4). Recent efforts have also applied this Z-score methodology to analyze data from the CDC's Social Vulnerability Index. Utilizing the four SPL-Theme values found in yearly SVI datasets, Z-scores can be calculated for each theme using a national mean and standard deviation. Z-score theme values per county are then added to provide an overall county-

based score, with higher values indicating a larger degree of social vulnerability.

Results & Conclusions: CEI data depicts yearly values extending to four, indicating a year with extreme data for all environmental variables. While analyzing trends in environmental z-scores, temperature appears to be a dominating factor in climate extremes. SVI data is currently in developmental phases; however, I anticipate higher-than-average levels of social vulnerability. After calculating the CEI and SVI for each county, the Extremes Vulnerability Index (EVI) can be created to provide measurable evidence of population vulnerability to climate extremes.

110. DEFINING THE MECHANISM OF TOXICITY OF LEPTOSPERMONE, A B-TRIKETONE HERBICIDE, TO AEDES AEGYPTI

Sarah E. McComic - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Charles Cantrel** - United States Department of Agriculture; **Werner J. Geldenhuys** - Department of Pharmaceuticals, College of Pharmacy, West Virginia University; **Rui Chen** - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Herbicide products are critical to the agricultural industry by preventing the development of weeds in fields of desirable crops. β-triketone herbicides target the 4-hydroxyphenyl pyruvate dioxygenase (HPPD) enzyme pathway that is critical for photosynthesis and carotenoid production, which is essential for overall plant health. The utility of HPPD inhibitors, such as β-triketones, has been well documented, but the insecticidal potential of HPPD and β-triketones remains understudied. Thus, the objective of this study was to characterize the mechanism of action, toxicity, and phenotypic effects of natural β-triketone HPPD inhibitors to insects. We first tested the toxicity of manuka oil which contains two primary fractions: leptospermone (a β-triketone) and a triketone, to Aedes aegypti mosquitoes. Leptospermone and the β-

triketone fraction were both topically toxic to Aedes aegypti (Rockefeller strain) with LD50 values of 158.4 ng/blood fed mosquito (95% C.I.: 124.3 - 204.3 ng, Hillslope: 1.77, r2: 0.84), and 155.5 ng/blood fed mosquito (95% C.I.: 137.5 - 176.3 ng, Hillslope: 5.2, r2: 0.92), respectively. Interestingly, less than 10% mortality was observed in Drosophila melanogaster and Apis mellifera when treated with high doses of leptospermone, indicating species specificity of leptospermone and potential species differences within HPPD enzymes. Extracellular central nervous system (CNS) recordings of Ae. aegypti were performed to test the influence of leptospermone and β-triketones to mosquito central nerve firing rates. Leptospermone did not alter spike discharge frequencies, suggesting the mechanism of toxicity may not be centered at the nervous system. Molecular modeling and in silico chemical overlays were performed and data indicate leptospermone has a high degree of chemical similarity to known carbonic anhydrase (CA) inhibitors. These modeling data led us to test the in vitro inhibitory potency of leptospermone to carbonic anhydrase enzyme activity from female Ae. aegypti midguts. Leptospermone inhibited CA enzyme activity with two-site binding activity with a high IC50 of 428.6 nM, and a low IC50 of 199.7 µM, which is highly potent considering it is a natural product. Finally, leptospermone significantly decreased midgut pH of Ae. aegypti which is similar to established CA inhibitors. These data suggest leptospermone induces toxicity to mosquitoes through carbonic anhydrase inhibition and implications the field of insecticide science will be discussed.

111. DOMAIN AND HISTOPATHOLOGY ADAPTATIONS-BASED CLASSIFICATION FOR MALIGNANCY GRADING SYSTEM

Se-woon Choe - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Vicky Mudeng** - College of Engineering, Kumoh National Institute of Technology; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Accurate proliferation rate quantification can be used to devise an appropriate treatment for breast cancer. Pathologists use breast tissue biopsy glass slides stained with hematoxylin and eosin to obtain grading information. However, this manual evaluation may lead to high costs and be ineffective because diagnosis depends on the facility and the pathologists' insights and experiences. Convolutional neural network acts as a computer-based observer to improve clinicians' capacity in grading breast cancer. Therefore, this study proposes a novel scheme for automatic breast cancer malignancy grading from invasive ductal carcinoma. The proposed classifiers implement multistage transfer learning incorporating domain and histopathologic transformations. Domain adaptation using pretrained models, such as InceptionResNetV2, InceptionV3, NASNet-Large, ResNet50, ResNet101, VGG19, and Xception, was applied to classify the ×40 magnification BreaKHis data set into eight classes. Subsequently, InceptionV3 and Xception, which contain the domain and histopathology pretrained weights, were determined to be the best for this study and used to categorize the Databiox database into grades 1, 2, or 3. To provide a comprehensive report, this study offered a patchless automated grading system for magnification-dependent and magnification-independent classifications. With an overall accuracy (means ± SD) of 90.17% ± 3.08% to 97.67% ± 1.09% and an F1 score of 0.9013 to 0.9760 for magnification-dependent classification, the classifiers in this work achieved outstanding performance. The proposed approach could be used for breast cancer grading systems in clinical settings.

112. EMBEDDED 3D PRINTING OF PERFUSABLE ALVEOLAR MODEL FOR LUNG DISEASE STUDIES

Bing Ren - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida; **Kyle Phillips** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Wenxuan Chai** - Department of Biostatistics, College of Medicine, University of Florida; **Justin K. Mensah-Mamfo** - Florida A&M University; **Sofia A Lugo** - University of Southern California; **Nazarius S. Lamango** - Florida A&M University; **Ite A. Offringa** - University of Southern California; **Lizi Wu** - Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida; **Yong Huang** - Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida

Despite the availability of conventional two-dimensional (2D) and animal models, there remains a critical need for innovative, physiologically relevant three-dimensional (3D) models. These models are essential to faithfully recapitulate the complex tissue architecture and cell-extracellular matrix (ECM) interactions observed in vivo, thus offering more accurate predictions of human responses. The goal of this research is to design and fabricate a 3D hydrogel-based human alveolar model using embedded 3D printing technology, which is valuable for studying viral infection and facilitating the development of new therapeutics. The 3D alveolar model we have designed has both an air channel and a liquid channel embedded in a thick hydrogel construct, resulting in an air-liquid interface (ALI) with diffusional permeability between the two channels. The air and fluid channels are seeded with human alveolar epithelial and endothelial cells, respectively, and then perfused with cell medium and humidified air to preserve dynamic flow conditions and key cellular components targeted by pathogens. The two perfusable channels are fabricated by directly extruding a sacrificial ink through a printing nozzle in a gelatin composite-based yield-stress support matrix, which locally liquefies upon the traverse of the printing nozzle and resolidifies in the wake of the nozzle, immobilizing the printed pattern in place. This embedded printing approach mitigates the effects of gravity by providing omnidirectional support on extruded filaments, allowing freeform

printing of complex channel structures in a 3D matrix. After the printing is completed, the matrix is enzymatically cross-linked, followed by the removal of the sacrificial pattern to allow the formation of perfusable channels. For the identification of new therapeutics, drug candidates can be perfused through the fluid channel and responses of alveolar epithelial cells can be evaluated. In comparison to conventional 2D cultures, this 3D alveolar model recapitulates the key morphological and functional characteristics of the alveolar ALI, which may enhance mechanistic insights into lung-related pathogenic diseases and facilitate the development of applicable therapeutics.

113. EXPLORING THE INFLUENCE OF THE MICROBIOME ON IMMUNE SYSTEM MATURATION

Troy R. Scoggins IV - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida; **Julia Flores** - Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; **Joseph P. Zackular** - Division of Protective Immunity, Children's Hospital of Philadelphia, Philadelphia, PA, & Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; **Michael A. Silverman** - Division of Infectious Disease, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA & Division of Protective Immunity, Children's Hospital of Philadelphia, Philadelphia, PA; **Boone M. Prentice** - Department of Chemistry, College of Liberal Arts and Sciences, University of Florida

In the arena of autoimmune diseases, regulatory T cells (Tregs) have emerged as pivotal players in maintaining immune homeostasis. Tregs are a specialized subset of T cells renowned for their ability to rein in immune responses, preventing autoimmunity and maintaining immune tolerance. Recent research has unveiled an intriguing facet of Treg biology: the capacity to generate peripheral regulatory T cells (pTregs) outside the thymus. These pTregs are produced in peripheral tissues and influenced by external factors, notably environmental signals like microbial metabolites and antigens. Evidence that microbiota are able to prevent certain autoimmune conditions underscores the need for understanding

the metabolic environment in the microbiome and its impact on immune system development.

A Pediatric Community (PedsCom) mouse model is designed to study the impact of bacteria on early-life immune system development. Their microbiome is composed of 9 select microbial species. A second mouse model is also used (CMCom) that is composed of a complex adult microbial community and is used as a positive control. These two models, along with a germ-free negative control model, were analyzed to study metabolic changes during weaning and their potential impact on the immune system. Murine ceca were harvested, sectioned at 12 □m thickness on a cryo-microtome (Leica Biosystems), and coated with a 1,5-diaminonaphthalene matrix layer using a robotic sprayer (HTX Technologies). MALDI imaging mass spectrometry was performed on a 7T solariX FT-ICR mass spectrometer (Bruker Daltonics).

Two timepoints are used for each model, pre-weaning (10 days) and post-weaning (5 weeks). This enables the study of metabolic changes during weaning that coincide with the induction of pTregs in PedsCom and CMCom mice. One pathway of interest is the metabolism of tryptophan to indole-containing molecules. Tryptophan is detected in all models, but most abundant in the post-weaning germ-free condition. In this model, tryptophan is roughly 2-fold more abundant when compared to the other models. This is hypothesized to be due to the increased metabolism of tryptophan by bacteria present in the PedsCom and CMCom models. This is reinforced by the distribution of indolelactic acid, an indole-containing tryptophan metabolite that is roughly 4-fold more abundant in PedsCom compared to germ-free, and roughly two-fold more abundant in CMCom compared to germ-free. These indole derivatives potentially promote Treg differentiation through their effect on dendritic cells. Overall, microbiota in the PedsCom and CMCom models metabolize amino acids generating molecules with an impact on immune system development.

114. FUNCTIONAL INTERACTIONS BETWEEN POTASSIUM-CHLORIDE COTRANSPORTER (KCC) AND INWARD RECTIFIER POTASSIUM (KIR) CHANNELS IN THE INSECT CENTRAL NERVOUS SYSTEM

Rui Chen - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida

Introduction: The K+/Cl- cotransporter (KCC) is the primary mechanism by which mature neurons maintain low intracellular chloride (Cl-) concentration in mammals, yet insect KCC remains understudied. GABAergic signaling is the cornerstone for fast synaptic inhibition of neural signaling in arthropods and thus, the γ-aminobutyric acid (GABA)-receptor-chloride-channel (GRCC) complex is a longstanding target for insecticides. Our data has shown that KCC is an essential ion transport system that mediates proper neurotransmission in the insect CNS by driving efflux of chloride ions to facilitate GRCC function.

Methods and Results: Genetic ablation or pharmacological inhibition of KCC with VU271 increased spike discharge frequency and significantly ($P<0.05$) reduced the CNS sensitivity to γ-aminobutyric acid. Further, simultaneous inhibition of KCC and ligand-gated chloride channel (LGCC) complex results in a significant ($P<0.001$) increase in CNS spontaneous activity over baseline firing rates that, taken together, supports functional coupling of KCC to LGCC function. Yet, gaps in knowledge remain regarding physiological drivers of KCC function and interactions of ion flux mechanisms upstream of GGCC in insects. Considering this, we employed electrophysiological and fluorescent microscopy techniques to further characterize KCC in the insect nervous system. Fluorescent microscopy indicated insect KCC2 is expressed in rdl neurons, which is the neuron type responsible for GABA-mediated neurotransmission, and are coexpressed with inward rectifier potassium (Kir) 2 channels. Coexpression of Kir2 and KCC suggested the possibility of functional coupling between these two K+ flux pathways. Indeed, neurophysiological recordings of the Drosophila CNS indicated that pretreatment of the Kir channel inhibitor VU041 at an EC10 followed by cotreament of Kir and

KCC inhibitors at an EC10 resulted in an increased of central firing rates by 40 ±11%, which is above the expected additive effect, supports functional coupling of Kir to KCC function. Further, pharmacological inhibition of KCC has been shown to lead to acute toxicity of mosquitoes that highlights the toxicological relevance of insect KCC.

Conclusions: These data expand current knowledge regarding the physiological roles of KCC and Kir channels in the insect nervous system by defining additional pathways that facilitate inhibitory neurotransmission through GGCC.

115. HARNESSING MACHINE-LEARNING TO DECIPHER LINKS BETWEEN ENVIRONMENTAL STRESSORS AND PER- AND POLYFLUOROALKYL SUBSTANCES IN COASTAL WATERS

Sunil Kumar - Department of Environmental Engineering Sciences, College of Engineering, University of Florida; **Sanneri E. Santiago Borrés** - 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; **Antarpreet Jutla** - Department of Engineering, College of Engineering

Widespread use of highly persistent, bioacumulative, and toxic per- and polyfluoroalkyl substances (PFAS) has led to environmental and public health concerns. Yet, it is not clear how changes in environmental conditions influence PFAS fate and transport in the environment. This study uses a machine-learning method, specifically Classification and Regression Trees (CART), to examine presence of PFAS in the Indian River Lagoon (IRL) in Brevard County, Florida. The research hypothesizes that the prevalence and emergence of PFAS are influenced by water quality and hydroclimatic variables and predicts their likelihood under different conditions. The study focuses on EPA-regulated PFAS, including perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorbutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), and perfluorooctane sulfonic acid (PFOS). It uses surface water samples from 17 IRL sites and considers environmental stressors such as

salinity, dissolved oxygen, pH, water temperature, precipitation, wind speed, and river discharge. The CART analysis identified salinity as a key factor linked to the presence of PFAS in the IRL, potentially associating it with PFAS sorption behaviors at the water-sediment interface. The study highlights the increased predictive accuracy for long-chain carboxylic PFAS compounds. The CART model showed an average sensitivity and specificity of approximately 87% and 57%, respectively. This research serves as a guide for future studies and takes a significant step towards providing valuable insights through machine learning for future assessments of PFAS exposure risks in aquatic environments and suggesting targeted monitoring and interventions based on identified stressors.

116. IDENTIFICATION OF PROTEOTOXIC AND PROTEOPROTECTIVE BACTERIA THAT NON-SPECIFICALLY AFFECT PROTEINS ASSOCIATED WITH NEURODEGENERATIVE DISEASES

Alyssa Walker - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Rohan Bhargava** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Michael Bucher** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Amanda S. Brust** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Cindy Voisine** - Department of Biology, Northeastern Illinois University; **Daniel Czyz** - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida

Neurodegenerative protein conformational diseases (PCDs), such as Alzheimer's, Parkinson's, and Huntington's, are a leading cause of death and disability worldwide and have no known cures or effective treatments. Emerging evidence suggests a role for the gut microbiota in the pathogenesis of neurodegenerative PCDs; however, the influence of specific bacteria on the culprit proteins associated with each of these diseases remains elusive, primarily due to the complexity of the microbiota. In the present study, we employed a single-strain screening approach to determine the effect of 229 unique bacterial isolates from

the Human Microbiome Project on the enhancement or suppression of disease-associated protein aggregation and the associated toxicity. Aggregation and the associated toxicity was assessed in Caenorhabditis elegans expressing human tau, Aβ1-42, α-synuclein, and polyglutamine tracts. Here, we present the first comprehensive characterization of the effect of the human microbiome on proteins associated with neurodegenerative diseases. Our results indicate that bacteria affect the aggregation of metastable proteins by modulating host proteostasis rather than selectively targeting specific disease-associated proteins. These results reveal bacteria that potentially influence the pathogenesis of PCDs and open new promising prevention and treatment opportunities by altering the abundance of beneficial and detrimental microbes.

117. IMPACT OF AGRICULTURAL PRACTICES AND GEOGRAPHIC LOCATION ON SOIL MICROBIOMES

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, University of Florida; **Jonathan Crabill** - University of Florida; **Bugil Choi**; **Charles B Appolon** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Laurel L. Dunn** - University of Georgia; **Keith R Schneider** - Department of Food Science and Human Nutrition, 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

Soils are the most biodiverse ecosystems on Earth, harboring microorganisms essential for nutrient cycling, maintaining soil fertility, and sequestering carbon. These microorganisms significantly influence the health of plants and animals, both directly and indirectly. Yet, the effects of agricultural practices and geographical conditions on soil microbial communities remain poorly understood. This study aimed to examine the soil microbiome under various treatments in Florida and Georgia, assessing how environmental factors and agricultural practices affect microbial populations. Over a period of 140 days, soil samples were exposed to six different treatments: a control (C), treated with E. coli (EC), enriched with poultry litter (PL), amended with poultry pellets (PP), a

control with onions grown in it (OC), and treated with poultry pellets while cultivating onions (OPP). Through 16S rRNA metagenomics sequencing, we analyzed the bacterial communities of the soils, assessing changes in microbial diversity, composition, and potential functionality across treatments and locations. Our findings indicated significant differences in microbial communities between the two regions, with poultry pellet treatments (PP and OPP) notably altering diversity and composition at both sites. Specifically, these treatments significantly boosted the relative abundance of Rhizobiales, a group of bacteria known for nitrogen-fixing, which converts atmospheric nitrogen into ammonia usable by plants. While other treatments had varied impacts on microbial diversity, none were as significant. The introduction of E. coli did not have a marked effect, likely due to the diversity and competitive dynamics of the soil microbiome. The research highlights the significant impact of geographic location and farming practices on microbial community behavior, underscoring the importance of incorporating microbial understanding into agricultural planning. This approach illuminates paths toward enhancing soil health, sustaining ecosystems, and boosting agricultural efficiency.

118. INITIATION OF IMMUNOMODULATORS OR ADVANCED THERAPIES COMPARED TO 5ASA ALONE NOT LINKED TO INCREASED RISK OF SERIOUS INFECTIONS IN THE MEDICARE IBD POPULATION

Ali Aalam - Department of Epidemiology, College of Public Health and Health Professions, University of Florida; **Tianze Jiao** - College of Pharmacy, University of Florida; **Arvin Daneshmand** - Department of Medicine, College of Medicine, University of Florida; **Fanxing Du** - College of Pharmacy, University of Florida; **Chelsea Salmon** - Department of Medicine, College of Medicine, University of Florida; **Tomas Potlach** - Department of Medicine, College of Medicine, University of Florida; **Angela Pham** - Department of Medicine, College of Medicine, University of Florida; **Ellen Zimmermann** - Department of Medicine, College of Medicine, University of Florida; **Naueen Chaudhry** - Department of Medicine, College of Medicine, University of Florida

Background: Advanced therapies for inflammatory bowel disease (IBD; immunomodulators, biologics, small molecule drugs) have been reported to increase risk of infection, particularly opportunistic infections, presumably by their immunosuppressive effects. The risk in older adults with IBD is incompletely understood and complicated by co-morbidities and other factors. We used a Medicare database and propensity score (PS) matching to estimate the risk of infection in patients treated with advanced therapies compared to patients treated with the non-immunosuppressive IBD therapy, mesalamine or 5ASA-derived agents.

Methods: We conducted a retrospective cohort study using Medicare Fee-For-Service claims data from 2012-2019. We included patients entering the cohort with an IBD diagnosis and newly prescribed IM or advanced therapies (IM/AT) or 5-ASA between 2012-2019. The date of first IM/AT or 5-ASA prescription was defined as the index date. We included patients aged >=65 with 1-year continuous enrollment of Medicare A, B and D. Patients who received IM/AT or 5-ASA within one year before the index date were excluded. We conducted PS-matching with 1:1 ratio between patients in the IM/AT and 5ASA group on demographic characteristics, comorbidities, and use of steroids as outlined in Table 1. Patients were followed until censoring due to the

outcome (infectious disease, included in Table 2.1), or 1-year follow-up, whichever occurred first. Cox proportional hazards model was used to estimate hazards ratios with 95% confidence intervals (CI) of 1-year infectious diseases adjusted for demographic characteristics, comorbidities, use of steroid, and duration of treated IBD.

Results: We identified 706 and 1,653 patients who initiated IM/AT and 5ASA group, respectively. After propensity score matching, there were 672 patients in both IM/AT and 5ASA groups. During 1-year follow up, 90 patients in the IM/AT group and 84 patients in the 5ASA group developed infectious diseases with an incidence rate of 14.5 and 13.5/100 person-years, respectively. In comparison between the IM/AT group and 5ASA group, the adjusted hazard ratio is 1.059 (95%CI: 0.786, 1.427).

Conclusions: After balancing patients' demographic characteristics and comorbidities, the initiation of the IM/AT in elderly patients with IBD correlates with a statistically insignificant, but slightly increased risk of infection.

119. MOLECULAR AND GENETIC CHARACTERIZATION OF USHER SYNDROME TYPE II

Katerina Anamisis - Department of Chemical Engineering, Undergraduate Research, College of Liberal Arts and Sciences, University of Florida

Usher syndrome is a rare genetic disease that affects both hearing and vision. This inherited autosomal recessive condition affects 4 to 17 per 100,000 people and accounts for about 50 percent of all hereditary deaf-blindness cases.

There are three distinct types of Usher syndrome, of which type II is the most common. Usher syndrome type II is caused by mutations of the USH2A gene, which encodes for the usherin protein. Usher syndrome is characterized as an autosomal recessive condition leading to usherin loss compromising protein structure resulting in progressive deterioration of photoreceptor function. Usherin protein plays important homeostatic roles for vision and hearing function where it localizes to photoreceptor cilium and cochlear hair cells. Phenotypically, this subtype of Usher

syndrome includes moderate to severe sensorineural hearing loss from birth and progressive loss of vision, prompting retinitis pigmentosa (RP). In RP, the photoreceptors progressively lose function, causing loss of peripheral vision that can lead to blindness by midlife and decreased night vision by adolescence.

Despite the varying severity of hearing loss, people with this condition can benefit from hearing aids and cochlear implants. As such, more attention is focused on developing treatments for the RP component of Usher syndrome. RP is usually diagnosed during late adolescence in people with Usher syndrome type II. To date, there is no cure for this disease. The large size of the USH2A gene makes gene therapy challenging as a potential treatment. Additionally, USH2A mutations often involve nonsense mutations where single base deletions induce a gene frameshift leading to introduction of premature stop codons. Such mutations in conjunction with large gene size limit therapeutic development options for these patients. As a result, a protein structure for usherin has not been solved and its exact molecular function has not been mechanistically elucidated.

Given the lack of basic biology understanding of usherin along with the high unmet need, my research mentor and I aim to establish usher syndrome patient derived cell lines to investigate usherin protein structure, molecular function, and cellular phenotyping analyses to understand its homeostatic roles in sensory function. This work will also serve as a foundation for identifying possible therapeutic targets capable of restoring usherin protein loss and function in vision and hearing contexts.

We will collect blood and 3 mm skin punch biopsy specimens from patients with Usher Syndrome Type II to generate dermal fibroblast cell lines, induced pluripotent stem cell lines, and differentiated hIPSCs (human induced pluripotent stem cell) lines to investigate molecular and cellular biology to characterize how USH2A gene mutations induce abnormal gene expression and molecular/cellular phenotypes associated with vision, hearing, and sensory loss. I will continue to conduct research in the Nano-Biomolecular Precision (Jain) Lab at UF on CRISPR-mediated

genome editing tool development for patients with Usher Syndrome Type II while also exploring alternative therapeutic approaches that may exist.

120. MOSQUITOES AS SENTINELS OF WILDLIFE DISEASE: CAN MOSQUITO BLOOD MEALS INNOVATE THE FIELD OF WILDLIFE DISEASE SURVEILLANCE?

Carson Torhorst - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Zoe White** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Sebastian Botero-Cañola** - Department of Wildlife Ecology and Conservation, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Lawrence Reeves** - Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida; **Nathan Burkett-Cadena** - Florida Medical Entomology Laboratory, Institute of Food and Agricultural 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

Environmental DNA (eDNA) is a promising technology with a diverse set of applications that have brought advancement to the fields of invasive species detection and management. The use of eDNA methods in xenosurveillance may provide a novel technique employing blood feeding organisms to sample and detect directly transmitted pathogens in wildlife species. If optimized, xenosurveillance could be used in routine surveillance efforts. This project sought to validate if torque teno sus virus (TTSuV), a directly transmitted porcine virus, could be detected in mosquito blood meals of feral swine. The objectives of this project were to 1) determine the percentage of mosquitoes feeding on feral swine, and 2) to establish if TTSuV could be detected in blood meals from a feral swine population known to circulate TTSuV in situ at a prevalence of greater than 40%. To this end, wild mosquitoes were collected from the University of Florida, Deluca Preserve from January to August 2022 and the blood meal host from the blood-engorged females (n=4557) was

determined through molecular DNA barcoding. Overall, 12 mosquito species from 5 genera fed on feral swine and feral swine DNA was present in 6.9% (n=314) of total identified blood meals. From the feral swine-fed mosquitoes, a subset of individuals (n=110) was tested for the presence of TTSuV using PCR methods. TTSuV was detected in the blood meals of the tested mosquitoes at a presence of 34.5% (n=38), similar to the prevalence recorded from direct sampling of feral swine. The detection of TTSuV from mosquito blood meals provides evidence for the use of xenosurveillance in the future detection and management of wildlife disease. Furthermore, this method may provide an alternative method for rapid surveillance in the case of outbreaks of emerging transboundary diseases such as African swine fever.

121. POTENCY OF PROBIOTICS DERIVED FROM HEALTHY CALVES FOR THE TREATMENT OF NEONATAL CALF DIARRHEA

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Miju Kim** - Kyung Hee University; **Peixin Fan** - Mississippi State University; **Sharath Rajeev** - University of Florida; **Sun Ae Kim** - Ewha Womans University; **J. Danny Driver** - Department of Animal Sciences, College of Agricultural and Life Sciences, University of Florida; **Klibs Galvao** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Neonatal calf diarrhea (NCD) remains a significant contributor to calf mortality within the first three weeks of life, prompting widespread antibiotic use with associated concerns about antimicrobial resistance and disruption of the calf gut microbiota. Recent research exploring NCD treatments targeting the gut microbiota dysbiosis has highlighted probiotic supplementation as a promising and safe strategy for gut homeostasis. However, varying treatment outcomes across studies suggest potential host-specific factors influencing treatment efficacy. In this study, we evaluated probiotics potency of Limosilactobacillus reuteri, formally known as Lactobacillus reuteri, strains isolated from healthy

neonatal calves. By completing comprehensive analyses, including whole-genome sequencing and in vitro assays, we identified nine L. reuteri strains, then these strains were administered to neonatal calves with diarrheal disease. Remarkably, calves treated with L. reuteri strains shed normal feces with restored gut microbiota and demonstrated healthy animal behavior. Leveraging a machine learning model, we evaluated microbiota profiles and found bacterial taxa associated with calf gut health that were elevated by L. reuteri administration. Our findings represent a crucial advancement towards sustainable, antibiotic-free alternatives for managing NCD, contributing significantly to global efforts in mitigating antimicrobial resistance and promoting overall animal health and welfare.

122. PROFESSIONAL DEVELOPMENT IN EMERGING PATHOGENS EDUCATION FOR SECONDARY SCIENCE TEACHERS

Matthew Phillips - 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

Collaborating to Advance Teaching and Learning of Science Educators and Students (CATALySES) was a 5-year, NIH-SEPA funded, teacher development program focused on Emerging Pathogen topics and research The program built on a novel model of scaffolded professional development where teachers partner with researchers to take Emerging Pathogens concepts and research practices to their classrooms. CATALySES developed teacher-leaders through biomedical research experiences; support in writing, testing, and publishing innovative traditional and technology-enhanced curricula; links with pre-service teachers; and continued interaction with researchers networked with regional communities of teacher-learners and their students. The CATALySES program was a partnership between the Center for Precollegiate Education and Training, the Emerging Pathogens Institute, the Clinical and Translational Science Institute (CTSI), the School of Teaching and Learning, and collaborating researchers from across the Health Science Center.

CATALySES hosted 4 cohorts, comprising 67 Florida teachers total, at a 2-week, on-campus institute. Additionally, 2 cohorts, comprising 19 teachers total, that attended the institute returned in-person or online for intensive, curriculum-development fellowships in active emerging pathogens research labs. The 67 teachers represented 21 counties and brought Emerging Pathogens knowledge back to 6,000+ students in their classrooms. During years 2 and 3, teacher cohorts completed pre-post surveys to assess teacher attitudes and science identity, use of technology, career awareness in STEM and Emerging Pathogens, and curriculum design expertise. Teachers came into the program with high leadership attitudes in science education and average-to-high technology use in their classrooms, but low curriculum design expertise and career awareness. Following the CATALySES institute, teachers reported an increase in curriculum design, career awareness in STEM and Emerging Pathogens-related fields, and knowledge of emerging pathogens.

The outcomes from CATALySES highlight the importance and impact of teacher-researcher interactions and networking in enhancing knowledge of STEM and emerging pathogens topics and career paths. Through UF research community support of teachers bringing this knowledge back to their classrooms and student attendance of summer STEM research programs, precollege students are better informed and prepared for the continuum of career paths in the science and health-related workforce.

123. REPELLENT ACTIVITY OF NATURAL PRODUCTS TO THE LONE STAR TICK, AMBLYOMMA AMERICANUM

Anais Le Mauff - Department of Entomology and Nematology, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Edmund Norris** - Center for Medical, Agricultural and Veterinary Entomology, USDA, Gainesville, FL 32608, USA; **Daniel Swale** - Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida

There is an increased need for the development of therapeutic agents that can prevent tick bites because the lone star tick, Amblyomma americanum is a competent vector for a variety of pathogenic organisms which cause significant disease in humans. Appropriate use of repellents is recommended by the US Center for Disease Control (CDC) to reduce or prevent tick bites and horizontal transmission of tick-borne pathogens to humans yet, the chemical diversity of tick repellents available to the consumer remains low. Thus, the goal of this study was to compare the efficacy of essential oils and naturally derived compounds as repellents to Am. americanum nymphs in three different bioassays: contact, spatial, and fingertip repellent assays. Concentration response curves after contact exposure to TCA led to a concentration required to repel 50% of ticks (EC50) of 5.6 µg/cm2, which was found to be 5- and 7-fold more active than DEET and nootkatone, respectively. For spatial bioassays, TCA was approximately 2-fold more active than DEET and nootkatone at 50 □g/cm2 but were not significantly different at 10 □g/cm2. In spatial assays, thyme and cassia were the most active compounds tested with 100% and 80% ticks repelled within 15 minutes of exposure respectively and was approximately 2-fold more effective than DEET at the same concentration. Using a finger-climbing assay, that quantified distance traveled on human skin, TCA, nootkatone, and DEET were equally effective and patchouli oil was the only natural oil that significantly repelled ticks. Interestingly, repellent potencies from the fingertip assay were negatively correlated to activity observed in the spatial or contact bioassays. These data suggest TCA is a promising candidate for a tick repellent that is comparable to commercialized tick repellents and

further, that assay design is critical for tick repellent development to ensure translation from lab to field.

124. SERVING UP DATA: TAILORING DATA DELIVERY TO RESEARCHER NEEDS

Benjamin J. Staley - Clinical and Translational Science Institute, College of Medicine, UF Health; **Herman Autore** - Clinical and Translational Science Institute, College of Medicine, University of Florida

Introduction: The Integrated Data Repository (IDR) serves as a common source of information for use by researchers, clinicians, educators and leaders across UF and the UF Health enterprise. The continuous flow of data between our clinical and research communities supports new scientific discoveries and patient quality and safety innovations as we move medicine and public health forward. Researchers can obtain datasets with rich details about patients and patient care to conduct various studies towards learning health system.

Methods: The IDR Research Services (IDR-RS) provides services of different complexity, each to suit researcher's needs. These can be likened to how one acquires food. At the most expedient level, there are self-service data tools, a sort of data "vending machine". Zero or low-cost services are the next option, similar to data "fast food". Next there are dedicated analysts, or "personal chefs", who provide service to specific researcher teams. Finally, there are on-demand requests, or data "custom meals". The organization fulfills these requests with a diverse staff of analysts and clinical liaisons. The analysts collect and release data, and the clinical liaisons help the analysts interpret medical information.

Results: The IDR-RS helps researchers develop models and pilot strategies for use in healthcare settings. In 2023, we supported 505 requests, including 406 IRB-approved data requests. This work resulted in numerous high-impact publications, grants, implementation of systems in healthcare settings, and improved health care. Some examples include near real time calculation of surgery risk, and increasing awareness for colorectal cancer screening using virtual human technology.

Discussion: An ongoing goal of IDR-RS is to provide quality outcomes by matching researchers with the most appropriate services based on their needs. The unit has recently committed itself to encouraging researchers to use Observational Medical Outcomes Partnership (OMOP) common data model, which allows standardizations and collaboration across institutions and is a faster method to obtain data from the IDR-RS.

125. SURVEILLANCE OF PATHOGENIC BACTERIA ON A FOOD MATRIX USING MACHINE-LEARNING-ENABLED PAPER CHROMOGENIC ARRAYS

Zhen Jia - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, University of Florida; **Boce Zhang** - Department of Food Science and Human Nutrition, Institute of Food and Agricultural Sciences, University of Florida

Foodborne outbreaks and illnesses are pervasive worldwide due to the consumption of foods contaminated with pathogens. Global food systems become complex and decentralized, advancing pathogen spread and cross-contamination. To bolster food safety and avert foodborne outbreaks, there is a pressing need for continuous monitoring of pathogens in food. However, the current detection approaches are cumbersome and destructive, lacking the capability to continuously monitor pathogens throughout the entire food system.

This study aimed to develop a nondestructive and nonculture-based paper chromogenic sensor array-machine learning (PCA-ML) system for simultaneously and continuously detecting multiple foodborne pathogens at low concentrations in foods.

A paper chromogenic array sensor - machine learning (PCA-ML) methodology, sensing concentrations of volatile organic compounds emitted on a species-specific basis by pathogens, was developed by streamlining dye selection, sensor fabrication, database construction, and machine learning analysis. PCA was fabricated by nine sensitive chemical dyes selected from 25 via principal component analysis. K-fold cross-validation was used to increase ML analysis robustness. The computational efficiency of the PCA-ML system was evaluated by continuous pathogen identification in ground chicken initially

contaminated at low bacterial concentrations (~1 to 3 log CFU/g). The PCA-ML system's capability to detect pathogens at loadings as low as 1 log CFU/g is comparable to most microbial testing systems approved by the Association of Official Analytical Chemists and the FDA.

The system enables noncontact, time-dependent, simultaneous monitoring of multiple pathogens (Listeria monocytogenes, Salmonella, and E. coli O157:H7) over time at either 4□C or 25□C. High identification accuracy (>90%) was achieved either at a high level (~3 log CFU/g) of initial pathogen contamination or at a low level (~1 log CFU/g).

The PCA-ML approach has great potential to be used in smart packaging, for extension to other food commodities and bacterial species, and for use in the food supply chain to monitor bacterial contamination continuously and nondestructively. Nondestructive microbial detection using PCA-ML can mitigate risks of foodborne illnesses and alleviate food waste burdens to help improve food system safety, security, and resilience.

Index of author by page

Notes

Notes